FREQUENCY-DEPENDENT SELECTION

AT THE AMYLASE LOCUS IN

DROSOPHILA MELANOGASTER

FREQUENCY-DEPENDENT SELECTION AT THE AMYLASE LOCUS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

A strain of *Drosophila melanogaster* deficient in amylase activity (*Amylase*^{null}) was isolated from a wild population of flies. The survivorship of *Amylase*^{null} homozygous flies is very low when the principal dietary carbohydrate source is starch. However, the survivorship of the *Amylase*^{null} genotype is comparable to the wild type when the dietary starch is replaced by glucose. In addition, the viability of the amylase-producing and *Amylase*^{null} strains is comparable, and very low, on a medium with no carbohydrates.

Furthermore, amylase-producing genotypes were shown to excrete enzymatically active amylase protein into the food medium. The excreted amylase causes the external breakdown of dietary starch to sugar. These results led to the following prediction: the viability of the *Amylase*^{null} genotype (fed on a starch rich diet) might increase in the presence of individuals which were amylase-producing. It was shown experimentally that such an increase in viability did in fact occur and that this increase was proportional to the number of amylase-producing flies present.

These results provide a unique example of a non-competitive inter-genotype interaction, and one where the underlying physiological and biochemical mechanism has been fully understood.

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II INTRODUCTION

Natural selection is simply the process of differential reproduction of individuals which differ in their genetic make-up. Differential reproduction may be predicted of an organism relative to another organism, of a genotype in relation to another genotype and of an allele in comparison to other alleles at the same locus. Population geneticists are primarily concerned with changes in allelic and genotypic frequencies in populations. Carriers of a given allele may contribute more progeny to the following generation than carriers of another allele; the former allele is then said to be favoured by natural selection and will increase in frequency in the population (Ayala and Campbell, 1974).

It has been known for almost two decades that large amounts of protein polymorphisms exist in natural populations (Lewontin and Hubby, 1966; Harris, 1966). The question then arises as to whether this variability, detected by electrophoretic screening, indeed reflects the differential adaptation which one would predict, i.e., an evolutionary geneticist is now faced with explaining a formidable amount of intraspecific genetic variation and its adaptive significance. The conventional response to this challenge has been to assume a correspondingly large amount of heterosis or heterozygous advantage (Berger, 1976). The belief in the importance of heterozygous advantage stems from the frequent

occurrence of "generalized" heterosis, in which hybrids (whether of whole chromosomal sets, individual chromosomes, or linked blocks of genes) are larger, more vigorous, and less variable than their parents (Gowen, 1952).

Thus, it seemed reasonable to expand the concept of heterosis to individual genes, which is supported by one clear example of single-gene heterozygous advantage, the sickle-cell trait in man (Allison, 1964). Heterosis can explain the amount of genetic variation but such a system assumes an intolerable genetic load. Moreover, heterotic selection generally assumes constant fitness. Experimental data, however, have indicated that selective values are not always constant and may vary with factors such as population density and/or the genotypic frequencies in a population.

The neutrality theory proposed by Kimura in 1968 and by King and Jukes in 1969 states that most of these protein variations are functionally equivalent. This theory provides a possible explanation of the widespread occurrence of enzyme polymorphisms. If we assume that genetic variants are selectively neutral, the genetic load is no longer a problem. However, the neutrality theory found little or no experimental evidence (Hickey, 1977; Scharloo et al, 1977; Hoorn, 1978 and others; see review by Clarke, 1979).

In a series of experiments on frequency-dependent selection in Drosophila melanogaster, Kojima and his co-workers

found that the relative survival of the three genotypes at the esterase-6 locus depended on their initial frequency; the rarer the genotype, the better are its chances of survival. He proposed frequency-dependent selection as " a possible general mechanism responsible for a large amount of the genic polymorphisms observed in natural populations." The essential feature of frequency-dependent selection is that fitnesses are not fixed but variable, and the values they take on vary as a function of the genotypic frequencies they characterize. In other words, fitness values vary so as to favour rare genotypes; for example, if the rarest genotype is the most fit, then the rare allele will increase in frequency but will not become fixed in the population. This is because as it becomes more common, the fitness of its carriers will decrease. This mechanism is attractive since it leads to a stable gene frequency equilibrium which does not involve the problems of genetic load (Gromko, 1977; De Benedictis, 1978b).

The number of experimental studies showing that the fitness of a genotype is affected by its frequency, has steadily increased in recent years. These experiments have been reviewed by Petit (1968, 1973), Ayala and Campbell (1974), De Benedictis (1978a, b) and Clarke (1979). Frequency-dependent selection has been demonstrated to be effective in maintaining variability in experimental populations of Drosophila by Lewontin (1955), Beardmore (1963), Ehrman (1966), Dawood and Strickberger (1964, 1969), Kojima et al (1967, 1969b, 1972), Huang et al (1971), Morgan (1976), Nassar et al (1974), Nassar (1979, 1980) and others; in sexual selection by Petit

(1951, 1958, 1967), Ehrman (1966, 1967), Spiess (1968, 1969) and others; and in interspecific competition by Budnik and Brncic (1974), Horsley et al (1979), Greenwood and Elton (predator-prey interaction, 1979), Arthur (1980), and Ayala (1971) whose experimental evidence led to the re-interpretation of the competitive exclusion principle.

It has been proposed that facilitation and/or interference are possible mechanisms for frequency-dependent selection observed in experimental populations (Weisbrot, 1966; Yarbrough and Kojima, 1967; Dawood and Strickberger, 1969). Inter- and intragenotypic facilitation or interference have been reported in Drosophila by Dawood and Strickberger (1969), Budnik and Brncic (1976), Bos (1979), Caligari (1980) and others. They all mentioned that biotic residues and/or nutrient depletion are possible mechanisms for facilitation or interference. However, none of these studies have reported or shown a specific way in which these biotic residues act, nor at which level they operate. Moreover, none of these studies have correlated facilitation or interference to a specific chemical or enzyme.

Thus, despite the success of these and other researchers in detecting frequency-dependent selection and the power of that selective mode in maintaining genetic variations, the mechanisms producing frequency-dependent selection are still unknown. This point has been illustrated by Clarke (1979) who in a review paper on the evolution of genetic diversity used as a sub-title for a brief review on frequency-dependent selection "Frequency-dependent selection whose mechanism is unknown."

No doubt more experimentation is needed to adequately assess the generality and the importance of the role of frequency-dependent selection in maintaining polymorphism. In addition, elaborate studies involving the biochemistry of the medium and the metabolic by-products should be initiated in order to fully understand how frequency-dependent selection operates.

The principal aim of this project was to investigate the mechanisms associated with frequency-dependent selection in Drosophila melanogaster at the amylase locus. Specific consideration was given to the biochemistry of the medium by reference to the amylase enzyme and its effect on the medium, in addition to the outcome of intraspecific competition.

III LITERATURE REVIEW

This section will focus on the following areas: interspecific and intraspecific interaction, genetic variability (polymorphisms) and how it is maintained in natural populations. The three major postulates, namely heterozygous advantage, the neutrality theory and frequency-dependent selection will be dealt with respectively. Finally, I will give a brief review of *Drosophila* amylases.

1) Interspecific Interaction

Populations of two or more species may or may not affect each other; if they do, the influence may be either beneficial (positive interaction) or harmful (negative interaction). In this section, the two essential ways in which individuals interact will be discussed.

a) Positive Interaction:

Commensalisms, co-operation and mutualisms are associations between two species or populations which result in positive effects on one or both species. These are ways in which organisms positively interact, and they are widespread in nature (Odum, 1971, Chapter 7).

Commensalism is the state in which one population benefits

while the other is unaffected. Commensalism represents a simple type of positive interaction and is perhaps the first step towards the development of mutually beneficial relationships. Many of the commensal animals are not host specific (Odum, 1971; Dale, 1957).

Co-operation takes place when both populations benefit from each other; however, it is not an obligatory association. One example of co-operation is the following: sterols are indispensable for insects, including Drosophila, and are used as precursors for the synthesis of ecdysone (moulting hormone), and as structural components of cell membranes. The viability of several Drosophila species raised on sterol mutants of yeast (Saccharomyces cerevisiae) is affected by the sterol content of the medium. Bos et al (1977) reported that Drosophila melanogaster and Drosophila simulans are both severely limited in their ability to grow in a monoculture on a sterol mutant of yeast (Saccharmoyces cerevisiae). However, when the larvae of both Drosophila species were grown together in a mixed culture their ability to survive was mutually increased.

In contrast to co-operation, mutualism is an association in which both populations benefit but one in which each population has become completely dependent upon the other. This relationship is also known as obligate symbiosis. Mutualisms are most likely to develop between organisms with widely different requirements, since organisms with similar requirements are more likely to get involved in negative interactions (Odum, 1971).

b) Negative Interaction:

Competition, predation, parasitisms and amensalisms are examples of negative interaction between individuals of two or more different species. A negative interaction has an adverse effect on the growth and survival of one or both of the two species. The negative effects, however, usually tend to be small where the interacting populations have had a common evolutionary past, since continued and severe depression of a prey or host population by the predator or parasite population will eventually lead to the extinction of one or both populations. Consequently, severe interactions are most frequently observed when there has been a large-scale or a sudden change in the ecosystem (Odum, 1971, Chapter 7).

Furthermore, Odum (1971) has stated that in the evolution of an ecosystem, negative interactions tend to be minimized in favour of positive interactions; thus, the survival of the positively interacting species will be enhanced. In addition, recent or new associations tend to develop severe negative co-actions more readily than older associations.

For example, Budnik and Brncic (1974, 1976) have shown that the larval viability of three species of the subgenus Sophophora, Drosophila melanogaster, Drosophila simulans and Drosophila willistoni, was not affected when raised in the presence of their own metabolic by-products, but their pre-adult viability was significantly reduced by the wastes of

Drosophila pavani. Furthermore, it is to be noted that the viability of Drosophila pavani larvae is seriously diminished when raised on a medium conditioned by itself or by Drosophila melanogaster.

The question whether two or more related species competing for the same limited resources can co-exist has been the subject of controversy for more than several decades. Gause's principle or " the principle of competitive exclusion" states that no two species are likely to be exactly identical in their efficiency to exploit any given resources, thus one of the two species will be at a relative advantage. Therefore, if competition occurs between these two species, the less efficient one will eventually be eliminated. Ayala (1969, 1970) studied two species of Drosophila, Drosophila pseudoobscura and Drosophila serrata, and observed their continued co-existence. The exclusion principle was insufficient in explaining the co-existence of the two Drosophila species, for the model predicted the elimination of one of the species. This led Ayala (1970) to argue that two species competing for the same limited resources may, under certain conditions, co-exist in a more or less stable equilibrium.

Based on additional experiments, Ayala (1971) pointed out that a necessary requirement for a stable equilibrium between two competing species is that their relative fitness must be frequency-dependent, and that the strong mutual depression of the populations results from interference.

2) Intraspecific Interactions

Competition between individuals of the same species occurs when common resources are in short supply; consequently, reductions in reproductive and survival rates take place. The effects and the degree of competition become greater with crowding and therefore are density-dependent.

a) Intraspecific Competition:

Intraspecific competition has been defined by Bakker (1961, 1969) as the " manifestation of the struggle for existence in which two or more organisms of the same species exert a disadvantageous influence upon each other because their more or less active demands exceed the immediate supply of their common resources ". Similar definitions have been given by Andrewartha and Birch (1954), Milne (1957) and Emlen (1973). All of these definitions include the notion of " harm " being exerted upon a number of individuals competing for resources which are common to both organisms, and in limited supply.

Competition (intra- or interspecific) is generally recognized to be one of the most important elements in natural selection. For example, rectangular survivorship, delayed reproduction, decreased clutch size, increased size of the offspring, parental care, mating systems, dispersed spacing and territoriality are all the consequence of intraspecific competition (Pianka, 1974). Furthermore, the efficient utilization of resources which are in short supply can also be the

end result of both intra- and interspecific competition (Pianka, 1974, p. 144)

Intraspecific competition is common and generally self-evident in plants. For example, young plants growing densely together must be thinned otherwise they will choke each other; this is very familiar to any farmer. In contrast, intraspecific competition in animals is harder to demonstrate, for animals are mobile and are thus difficult to observe over long periods of time. Furthermore, their food resources often vary, therefore avoiding each other would be selected for (Colinvaux, 1973).

Since competition favours the evolutionary divergence of populations, it is also advantageous for individuals within a population to diverge from each other. Yet intraspecific divergence is not as frequently observed as divergence between species. Ricklefs (1976, p. 215) gives two basic reasons for the lack of observable intraspecific divergence. He pointed out that the evidence for divergence between populations is preserved whereas the lack of it leads to extinction. Similarly, if individuals in a population do not diverge from one another, some individuals will succumb because they fared competition poorly; nonetheless, the population itself will persist. Secondly, a more important reason for lack of divergence within a population is that there is no simple genetic basis for divergence. Different populations have independent gene pools thus genetic difference will accumulate rapidly, but in the same population due to sexual reproduction, individuals at least potentially share most of their

genes. Nevertheless, differences may occur within the same population in the form of sexual dimorphism (Ricklefs, 1976, p.215).

One could always measure intraspecific competition in terms of a density-dependent process. For example, intraspecific competition for food may result in density-dependent mortality. However, as early as 1954, Nicholson recognized two extreme forms of intraspecific competition, known as "contest" and "scramble" competition. In "contest" competition the successful individual obtains all that it requires but the unsuccessful one procures insufficient resources or space for survival or reproduction. An often-stated theoretical example is the competition between solitary wasps for a limited number of nest holes. A contest for 100 nest holes with 100 or fewer competitors poses no problem, as there is no shortage. However, with 200 competitors only 100 can find nest holes and in consequence 50% will fail to breed (Varley et al, 1974, p. 26).

Various species of insects can be reared in small vials under closely-monitored conditions. Rearing is usually performed in an incubator which is maintained at a constant temperature, light and humidity level; these conditions are obviously artificial. Nevertheless, these experiments permit the control of all variables except the population number. Consequently, under these conditions intraspecific (or interspecific) competition for food or space becomes more important and can thus be studied.

Using the above as a rationale, Crombie (1945) cultured Rhizoperth dominica (small Bootry-child beetle), beginning with a

single pair of beetles in 10 g of wheat grains. These grains had been cracked slightly by compression; eggs were laid in these cracks. Every seven days the grains were sieved off, their weight was restored to 10 g with the addition of freshly cracked grains, and all faecal matter was discarded. Thus, the food resources were approximately constant. Dead and live beetles were counted every two weeks; after approximately 200 days, the population reached a steady state with a mean value of 338 beetles. At this point in time, the number of newly hatched beetles is equal to the number of the dead, and only the fittest beetles survive.

In "scramble" competition, however, the available resources are shared among all the competing animals. When the individuals are identical and the habitat is uniform, the sharing would be equal; the mortality rate would rise immediately from 0% to 100% when the resources per individual become insufficient for survival.

Bakker (1961) reported that when a known number of Drosophila melanogaster larvae were put to compete on an agar surface with known amounts of yeast on which the larvae fed, by varying the numbers of larvae on a fixed quantity of food or by varying the amount of food for a fixed number of larvae, the results were identical. In other words, it was the quantity of yeast per larva that was critical for survival.

b) Facilitation or Interference:

Changes in the pre-adult viability of Drosophila have been

reported to take place because of changes in larval density. Furthermore, pre-adult viability can also be affected by the presence of larvae of other genotypes (Lewontin, 1955; Bakker, 1961; Dawood and Strickberger, 1969; Bos, 1979 and others). Many of these experiments indicated that a significant portion of the observed effects on viability, whether facilitation or interference, are probably caused by substances secreted by larvae into the common culture medium.

In 1950, Chiang and Hodson pointed out that larval viability in Drosophila melanogaster was affected by the larval density although each larva was receiving an equal amount of food at all densities used. This led them to suggest that this effect was caused by waste products excreted by the larvae into the medium. Similar reasoning was offered for the observed interference by Lewontin (1955), Bakker (1961), Budnik and Brncic (1976), and for the observed facilitation by Bos et al (1977), Bos (1979) and others.

In order to demonstrate that this effect can be caused by metabolic by-products and not merely by direct physical contact between the larvae, a technique was adopted by Weisbrot (1966) which permits one to measure larval viability on a medium containing by-products excreted by other larvae which are no longer present or active in the medium. The essential feature of this technique consists of " conditioning " the medium by using larvae of a given genotype for a given period of time, then physically removing them; in order to ensure total inactivity of

the remaining larvae, the conditioned medium was frozen for a few hours.

Bos (1979) has reported that mutual facilitation did occur between two genotypes of Drosophila melanogaster. These two genotypes (ebony and F-spa) have low viability when raised in a monoculture on the erg-2, sterol mutant yeast, Saccharomyces cerevisiae; however, when the larvae of both Drosophila melanogaster genotypes were raised together in a mixed culture, their viability each increased significantly.

These results do resemble the data obtained by Huang et al (1971) and Kojima and Huang (1972) who compared the viabilities of different combinations of genotypes at the esterase-6 locus in Drosophila melanogaster. They reported that in some cases the conditioning of the medium by larvae of a given genotype increased the viability of larvae of some other genotypes. They postulated that this " facilitation " was probably due to favourable metabolic products. Furthermore, they pointed out that the possibility of a favourable effect on the physical texture of the medium cannot be excluded. However, with other genotypic combinations, conditioning of the medium has an antagonistic effect; whether or not this is due to a depletion of resources and/or to harmful metabolic products which are involved in these cases of interference, it cannot be decided based on these experimental data.

Mutual facilitation by biotic by-products is not restricted to insects such as Drosophila. The situation is

similar to the phenomenon of syntrophism which involves mutual crossfeeding due to the utilization of diffusible metabolic by-products by microbial mutants with different metabolic blocks (Braun, 1966). Furthermore, mutual facilitation occurring between genotypes provides a mechanism for the maintenance of genetic polymorphism (Lewontin, 1955; Beardmore, 1963). Although these explanations are correct, that the by-products are responsible for increasing (facilitation) or decreasing (interference) larval viability, no one has offered an explanation indicating how the by-product might negatively affect one genotype while enhancing the viability of another or showing at which level these excreted products operate. Moreover, no one has been able to determine if the degree of facilitation or interference is frequency-dependent or not.

c) The Effect of Density-Dependent Selection:

There are various ways in which genotypic fitnesses can depend on the population size; it is known that some negative density-dependent factors do operate at high densities otherwise density would increase indefinitely. On the other hand, there are also important positive density-dependent factors which operate at low densities, a phenomenon which is often referred to as the Allee effect. Allee et al (1949) and Andrewartha and Birch (1954) have pointed out that many populations have an apparent minimal population size, an effective number below which they decline to extinction.

There is a considerable amount of evidence which indicates that both overcrowding and undercrowding can be detrimental to a population. As early as 1911, Howard and Fiske were able to distinguish three kinds of mortality factors (density-dependence, density-independence and predators) when they studied the gypsy moth and the brown-tailed moth.

There are various reasons for the Allee effect; some of them are: organisms grouped together can often better withstand adverse environmental conditions and attacks by predators, they are better able to locate food, and more importantly bisexual organisms must be sufficiently abundant to encounter a mate (Allee et al, 1949; Andrewartha and Birch, 1954; Lewis and Taylor, 1967). Also, Lewontin (1955), and Lewontin and Matsuo (1963) found that Drosophila melanogaster genotypic viabilities are often low at extreme larval densities with a maximum viability at an intermediate density. They too pointed out that the genotypes differed not only in their optimal densities but also in their maximum viabilities. In addition, Alvarez et al (1979) have shown that the larva-to-adult viability of different genotypes of Drosophila melanogaster was primarily dependent on both larval density and the genotypic frequencies in the population. A number of mathematical models have been proposed and investigated in which fitnesses are arbitrary functions of the changing population size (Asmussen, 1979; Slatkin, 1979).

3) How is Genetic Variability Maintained in a Natural Population ?

a) Protein Polymorphisms and their Adaptive Significance:

The application of the gel electrophoretic technique to population genetics by Harris (1966) and by Lewontin and Hubby (1966) revealed an unexpected wealth of genetic variation in natural populations. This discovery brought about what Lewontin (1974) called the " paradox of variation ".

Since that breakthrough, the technique has been used to obtain evidence for the existence of protein polymorphisms in a large variety of organisms such as human red blood cell antigens (Lewontin, 1967), field mice, lizards, horseshoe crabs (Selander et al, 1970) and Drosophila (Lewontin and Hubby, 1966 and others). However, one has to point out that electrophoresis cannot detect all variability because many amino acid substitutions do not change the electrical charge of proteins. Nevertheless, these detectable genetic variations which are surprisingly common, do exist. The question arises as to whether this variability reflects differential adaptation which one would expect. In addition, if these protein variants do indeed reflect differential adaptation, then one might predict that the amount of such variations would most logically correlate with environmental diversity. In other words, one would predict that the number of available niches would in some way dictate the level of variability. Various authors tested the above prediction; typical results were reported by Somer and Soulé (1974) who estimated the level of protein variability in tropic, temperate and arctic

species of fish. They found that fish species in tropical areas are more polymorphic than the temperate species. Thus, in this study protein polymorphism is seen to correlate with a region of low thermal diversity. Furthermore, a variety of other studies have reported correlations between the degree of protein variability and latitude (Johnson, 1974b).

Despite this correlation, these results are difficult to interpret, for example, it is not clear whether variability in the tropical areas is due to temperature or to the variation of some other factors such as resource diversity. Therefore, a variety of studies have readdressed this question by examining the level of protein variation at specific loci in habitats differing in ways known to affect the functioning of the enzymes specified by these loci.

Presently, there is a considerable amount of data on specific enzymatic systems; a typical example is the enzyme, alcohol dehydrogenase (ADH), in Drosophila melanogaster. Vigue and Johnson (1973) have reported that there is a marked latitudinal cline along the eastern seaboard of the United States, with Florida being basically monomorphic and New York highly heterozygous. However, ADH has been found to be linked to a small inversion which exhibits the same latitudinal cline in frequency. Consequently, it is not clear whether the linked inversion or the ADH is the target of the presumptive differential selection. Furthermore, the function of the ADH enzyme (Gibson, 1970) in Drosophila not only catabolizes ethanol as it was generally

assumed but also a variety of other physiological processes which include retinene to retinol and glyceraldehyde to glycerol (Johnson, 1976).

A few loci have been studied in which the physiological role seems to be clear; these loci are responsible for maintaining NAD/NADH oxidation-reduction potentials in the cell (Redox): in invertebrates, the α-glycerophosphate dehydrogenase (α-GPD) and in vertebrates lactate dehydrogenase (LDH). Merritt (1972) has shown that at the LDH locus in freshwater fish, there is a correlation between the water temperature and the degree of polymorphisms. Moreover, Johnson (1974b) has reported that the α-GPD loci of Drosophila are generally far more polymorphic in temperate regions than in tropical ones. One has to point out that the changes in temperature affect the LDH and α-GPD modulating enzymes along with fatty acid desaturation and energy (ATP/ADP) modulating enzymes.

Thus, up to now and in at least a few cases, the degree of protein polymorphism is seen to correlate with the environment, as an adaptive hypothesis would predict. However, this does not establish a clear cut case of a cause-effect relationship because the possibility that selection could be acting at other loci (genetic background) cannot be excluded. Nevertheless, most investigators have concluded that the observed polymorphisms are adaptive and that they are maintained primarily by some form of balancing selection.

Three major theories were proposed to explain this formidable amount of intraspecific genetic variation. These theories were heterozygous advantage, the neutrality theory and frequency-dependent selection.

b) Heterozygous Advantage: " Heterosis "

The term " heterosis " was coined by Shull's in 1916 to describe the vigour of (F_1) first generation hybrids (Spiess, 1977, pp. 10-11). The term " heterosis " is often used by geneticists as applied to heterozygotes which display some increased vigour or the excess of a trait beyond the expectation of pure homozygous parents. The aim of this subsection is not to review the vast amount of literature on heterotic selection, but rather to provide some insight on the concept and on the nature of this mode of selection.

Heterosis can explain the amount of genetic variation but such a system assumes an intolerable genetic load on the population (Kimura and Crow, 1964); furthermore, this system assumes constant fitness. Experimental evidence, however, points out that the selective value of a given genotype is often a function of its frequency in the population (review: Ayala and Campbell, 1974; Clarke, 1979).

Nevertheless, the above theory has been supported by two basic arguments and one clear example, as mentioned previously, i.e., sickle-cell anemia (Allison, 1964). The first argument to be considered is that hybrid molecules are functionally superior to

their homologous non-hybrid multimers. Fincham (1972) pointed out that there is a striking resemblance between heterozygous advantage and intracistronic complementation, in which two or more defective polypeptides combine to produce dimer or polymer enzymes. Fincham went on to argue that this kind of complementation may be a general cause of enzymatic polymorphisms.

The second argument states that heterozygotes are better buffered than homozygotes. In 1954, Lerner and Haldane both suggested that heterozygosity is in itself advantageous because it provides a buffer against environmental or genetic disturbances of the phenotype. Although heterozygotes may not be superior in a stable environment, they could be at a definite advantage when the environment varies in space or time. This is because a heterozygote will gain from having two or even three enzymes instead of one as in a homozygote.

Although the above arguments are persuasive, there are equally powerful counter-arguments and experimental evidence suggesting that heterozygous advantage does not provide a satisfactory, general explanation for the widespread polymorphism. In the first place, the argument that hybrid molecules make better enzymes, as a general explanation of polymorphism is unacceptable; monomeric enzymes are, on the average, more often polymorphic than those composed of dimers or higher polymers (Harris et al, 1977; Ward, 1977). Furthermore, Berger (1974) subjected this argument to a direct test; by using the highly polymorphic esterase-5 locus of Drosophila pseudoobscura, he found no evidence of hybrid

multimerous advantage. Berger's work (1974) indicated that in general, hybrid multimers were not superior to their homologous counterparts, and in several cases they were even found to be inferior. Thus, the available molecular evidence argues against the hypothesis of heterozygous advantage rather than for it. Secondly, the argument that heterozygotes are better buffered cannot be accepted because it is dependent upon the previous argument. Furthermore, doubts about the efficacy of heterozygous advantage have been greatly intensified by the fact that genetic variability does not seem to be dramatically reduced in self-fertilizing species (Selander, 1976) nor in haploid bacteria (Milkman, 1975), neither of which contain many heterozygotes.

Therefore, there is little direct evidence indicating that the widespread enzyme polymorphisms can be explained by the theory of heterozygous advantage.

c) The Neutrality Theory:

The neutrality theory which has its roots in the thinking of King and Jukes (1969) and Kimura and Ohta (1971), postulates that most of these protein variants are functionally equivalent. Contrary to the theory of heterozygous advantage, in the neutrality theory, the genetic load is no longer a problem, however, the concept in its strongest form, treats relative selective values as if they were absolute fitnesses, a procedure which is certainly not generally valid.

The neutrality hypothesis recognizes the importance of

natural selection but points out that evolution, at the molecular level, mostly occurs through random genetic drift. In addition, the neutrality hypothesis proposes that at most loci there are a number of mutants which are functionally equivalent with respect to adaptation. Therefore, they do not differ in their adaptiveness to the environment (Kimura and Ohta, 1971).

There have been numerous attempts to discriminate between the neutrality and selective theories. For example, Ayala et al (1974a) and Ayala (1974, 1977) tested the neutrality theory. The basis of their test was to compare the configurations of allelic frequencies between different species in order to assess whether these configurations come about by an independent sequence of random events or not, since according to the neutrality theory, genetic frequencies change at random due to the stochastic events of sampling from the gene pool of a population, from one generation to the next. The results obtained led Ayala (1977) to suggest that the genetic patterns in Drosophila willistoni cannot be explained by a random process such as genetic drift. Therefore they concluded that protein variants are subject to natural selection. Furthermore, one has to add that patterns of allelic distribution are found both to support and reject the neutrality theory (Ayala, 1977).

In addition, it has been pointed out by Ricklefs (1976, pp. 306-307) that the level of polymorphisms seems to be unrelated to the rate of evolution. For example, it is just as high in the horseshoe crab whose features have changed very little

over the past few hundred million years, as it is in rodents. Furthermore, some groups of Drosophila, for example, appear to be highly polymorphic while other animal groups show relatively little polymorphism (such as frogs and lizards), although it is not understood why such a difference exists.

Laboratory experiments have shown that, in general, whenever enzymes or other loci have been subjected to selective pressure directly related to their particular function, selection has been detected. For example, in Drosophila melanogaster experiments involving appropriate environmental stresses have given evidence of selective differences between amylase allozymes (Hickey, 1977; Scharloo et al, 1977; Hoorn, 1978); esterase, (Ghandi, 1978); alcohol dehydrogenase in Drosophila melanogaster, (Morgan, 1975; Van Delden et al, 1978). Yamazaki (1971) studied esterase in Drosophila pseudoobscura but found no evidence of selection; it is clear, however, that in his study the flies were not specifically stressed (Ayala and Campbell, 1974). Furthermore, among all of these studies there has not yet been any clear example of heterozygous advantage (Jones and Yamazaki, 1974; Wills et al, 1975).

Due to the fact that neither the theory of heterozygous advantage nor the neutrality theory could account for the widespread polymorphisms, a third hypothesis namely frequency-dependent selection was proposed.

d) Frequency-Dependent Selection:

It has long been known that frequency-dependent selection

favouring rare genotypes, is potentially capable of maintaining polymorphisms. As early as 1930, Fisher pointed out that adaptive values might be inversely related to genotypic frequencies in certain cases, thus leading to stable polymorphisms. However, only in the last decade has evidence accumulated indicating that this kind of selection is a major force in promoting and maintaining genetic diversity in natural populations.

The two essential features of frequency-dependent selection are: firstly, that fitnesses are not fixed but variable, and that the value they take on vary as a function of the frequency of the genotypes they characterize, and secondly, that the fitness values vary so as to favour rare genotypes and become approximately equal as an intermediate frequency is approached. This second feature of frequency-dependent selection ensures the maintenance of a balanced polymorphism without the problem of genetic load at equilibrium. Furthermore, the common description of frequency-dependent selection is that fitnesses vary, favouring rare genotypes. For example, if the rarest genotype is the most fit then the rare allele will increase in frequency but will not become fixed in the population because as it becomes more common, the fitness of its carriers will decrease. This mechanism is attractive since it leads to a stable gene frequency equilibrium which does not involve the problems of genetic load.

In this section, the following points will be dealt with:

(i) a review of various experimental studies dealing with

frequency-dependent selection.

(ii) apostatic selection

(iii) rare male advantage

(i) Experimental Studies on Frequency-Dependent Selection:

The first experimental evidence indicating that the fitness of a genotype was related to its relative frequency, was encountered by Wright and Dobzhansky in 1946. Their studies on inversion polymorphisms in natural and laboratory populations of Drosophila pseudoobscura have shown that the frequencies of two chromosomal arrangements oscillate throughout the seasons. They suggested that selective values might vary with changes in the composition of the population, however, they considered this explanation unlikely. Levene et al (1954) studied experimental populations of Drosophila pseudoobscura with three different genic arrangements. Their results have shown that the adaptive values of the genotypes depended upon which other genotypes were present, and in what frequencies in the population. They concluded that these results are not surprising, since other organisms are part of the total environment.

In a series of experiments carried out in Drosophila by Kojima and his co-workers (Kojima et al, 1967, 1969, 1971 and 1972), the generality of the phenomenon of frequency-dependent selection in experimental populations was ascertained. Tobari and Kojima (1967) studied changes in the frequencies of two inversions in both chromosomes 2 and 3 in sixteen experimental

cage populations. They found that the populations started with different initial frequencies always converged towards the same stable equilibrium. An analysis of their results indicated that fitnesses are strongly frequency-dependent. Furthermore, they pointed out that when the frequency of an inversion is below its equilibrium level, homozygotes for that inversion have the highest relative fitness and vice versa, with the heterozygotes having generally an intermediate fitness between the two homozygotes.

Ayala and Campbell (1974) criticized the method used in the above study to calculate the genotypic fitnesses, pointing out that Tobari and Kojima's method (1967) " would be appropriate if there were no differences in fecundity between the genotypes; that is, if zygotes were formed precisely in the Hardy-Weinberg frequencies expected from the genic (or inversion) frequencies in the parental generation. " Thus, in such a case, the fitness would depend solely on the egg-to-adult viability. In a later study, Kojima and Tobari (1969b) have shown that the egg-to-adult viability was indeed frequency-dependent in Drosophila ananassae. Furthermore, in their studies of polymorphism at the alcohol dehydrogenase and esterase-6 loci in Drosophila melanogaster, Kojima and Tobari (1967, 1969b) reported that the egg-to-adult viabilities are inversely related to the genotypic frequency.

In two further studies, Huang et al (1971) and Kojima and Huang (1972) attempted to elucidate the mechanism of frequency-dependent selection. The method employed was similar to the one suggested by Weisbrot (1966) which is as follows: allow larvae

of a known genotype to reach pupation on a measured amount of medium; the pupae are then removed and the remaining larvae are killed by freezing. Larvae of a known genotype are then transferred to this " conditioned " medium and their viabilities are measured. The results obtained by Huang et al (1971) and Kojima and Huang (1972) indicated that the relative viability of a given genotype was lowest when raised in media conditioned by larvae of the same genotype (interference), and highest when conditioned by larvae of a different genotype (facilitation). In addition, the viability of homozygous larvae increased as the frequency of their allele among the conditioning larvae decreased and vice versa. Thus, it is clearly seen that the conditioning of the medium by larvae of a given genotype makes the medium less favourable to larvae of the same genotype. They concluded that this is due to either the depletion of some required nutrients or to the production of diffusible metabolites. Other instances of inter- or intragenotypic facilitation or interference have been reported in Drosophila by Dawood and Strickberger (1969), Budnik and Brncic (1976), Bos (1979), Caligari (1980) and others. They all argued that biotic residues and/or nutrient depletion are possible mechanisms for facilitation or interference.

The number of experimental studies showing that the fitness of a genotype is affected by its frequency, has steadily increased in recent years. Many experiments have shown that larval viabilities depend on genotypic frequency as well as on the total density in Drosophila, such as those performed by Lewontin (1955, 1963), Birley and Beardmore (1977), Dawood (1969),

Anxolabéhère (1976, 1980), Snyder and Ayala (1979), Nassar (1980) and others; in Tribolium by Sokal and Huber (1963) and Sokal and Karten (1964); in houseflies by Taylor and Sokal (1973; and even in E. coli by Adams et al (1979). It is often seen that there is an inverse relationship between genotypic fitnesses and frequency.

(ii) Apostatic Selection:

Clarke (1962) has proposed the term " apostatic selection " to describe the situation in which natural selection favours rare phenotypes. Phenotypic polymorphisms occur in natural populations if predators consistently prey on the most common type among their prey, then the rare phenotype will have a selective advantage over the common form. Such behavior therefore tends to maintain balanced polymorphisms in the prey population because the selective value of a given phenotype varies inversely with its frequency in the prey population (Ayala and Campbell, 1974).

The first clear evidence of apostatic selection was obtained by Popham (1941, 1942). He found that Ciprinid rudds eat a greater number of individuals of the most common colour type among their beetle prey than one would expect if they were to eat them in proportion to their relative numbers. Furthermore, Tinbergen (1960) studied the effects of bird predation on insect larvae. He found that titmice ate a greater number of common species and a disproportionately smaller number of a rare one. This observation led him to postulate the well-known " search image hypothesis ". A similar observation of this phenomenon has

been reported by Mook et al (1960), Allen (1972), Horsley et al (1979) and others. In most of the reported cases, the predators tended to eat disproportionately more of the common forms of their prey.

The remarkable examples of mimicry found among prey species of butterflies, such as the swallowtails, Papilio dardanus and Papilio memnon, testify to the effectiveness of selection by predators (Clarke and Sheppard, 1971). Furthermore, in the polymorphic mimic Pseudacrea eurytus, the frequencies of the mimetic are found to adjust to the number of their respective models (Sheppard, 1959). This adjustment implies that the selection on the polymorphism is in fact frequency-dependent. In addition, an interesting extension of the phenomenon of frequency-dependence in predator-prey interactions has been put forward by Paulson (1973). He pointed out that prey species, especially the quick learners, can in fact exert frequency-dependent selection on their predators. He argued that prey may come to associate a particular visual, olfactory or behavioral pattern with the predator and consequently learn to avoid it. Thus, any predator that departs from the norm will be at a selective advantage. Paulson (1973) pointed out that avian predators hunting relatively intelligent and visually acute prey tend to be more often polymorphic for colour than those hunting unintelligent and dim-sighted prey.

Finally, the widespread occurrence of apostatic selection is generally accepted although contradictory evidence exists about

its occurrence at different densities (Holling, 1965; Allen, 1972). Nevertheless, whenever it occurs it will contribute to the maintenance of smell, pattern or colour polymorphisms among the prey populations. It may also act to promote diversity of behaviour (Humphries and Driver, 1967) and morphology (Clarke and Sheppard, 1962).

(iii) Frequency-Dependent Sexual Selection (Rare Male Advantage):

Frequency-dependent sexual selection is defined as any departure from random mating which is a function of the frequencies of the genotypes involved. This phenomenon (rare male advantage) was first discovered by Petit (1951) in Drosophila melanogaster. She found that when the frequency of the Bar males in the population was smaller than 60%, the number of their matings per male was nearly twice as great than when their frequency was greater than 80% (Petit, 1951, 1958).

Ehrman et al (1965) using observation chambers, found that the mating success of two types of Drosophila pseudoobscura males is inversely related to their frequencies. When equal numbers of the two karyotypes were used, mating was achieved independently of the karyotype. However, when one karyotype is less frequent than the other, rare males were observed to participate in a disproportionately larger number of matings. Many experiments have shown that the mating of a rare genotype is frequency-dependent favouring rare types. In Drosophila it seems to be a ubiquitous phenomenon (Ayala, 1972).
It has been reported in Drosophila melanogaster by Bundgaard and

Christiansen (1972), Petit (1951, 1958) and Rasmuson and Ljung (1973); in Drosophila pseudoobscura by Spiess (1968) and Ehrman (1967); in Drosophila persimilis by Spiess (1969); in Drosophila immigrans by Ehrman (1972), and in other Drosophila species, in fact, it seemed to occur in every species tested (Ayala and Campbell, 1974). Furthermore, it has been reported that this phenomenon takes place between strains differing in their geographic origin (Ehrman, 1966 and 1972); in chromosomal arrangements (Spiess, 1969 and 1970) and even between strains carrying different alleles at a single locus (Bundgaard and Christiansen, 1972; Ehrman, 1966, 1970 and others).

In a series of experiments, Ehrman (1966, 1969, 1972) investigated the mechanism involved in the rare male mating advantage. By using a double observation chamber separated by two layers of cheesecloth which form the floor of one chamber and the roof of the other, an electric blower blowing a soft air current from one chamber to the other, was placed on one chamber. Ehrman placed five pairs of strain A and twenty pairs of strain B in the proximal chamber, and fifteen pairs of strain A in the distal chamber. She found that whenever the air flowed from the distal chamber towards the proximal chamber, the A males did not exhibit mating advantage; however, when the air flowed from the proximal chamber to the distal chamber, the A males exhibited mating advantage. These results clearly suggest that the stimulus involved is olfactory. Nevertheless, some experiments gave results which were inconsistent with the notion that the stimulus involved in frequency-dependent sexual selection is exclusively chemical

(Petit, 1973).

Finally, the generality of the phenomenon led one to question its validity. For example, when one considers the whole fly genome, every Drosophila individual has a unique set of genes and is thus rare. Furthermore, it is not likely that every single genic difference could be recognized by the flies. Therefore, the possibility that the phenomenon of rare male mating advantage is merely an experimental artifact whose causes are not yet clear, cannot be ruled out. Burley (unpublished) has evaluated the rare-male literature; she argued that females should have type preference. Whereas, evidence for rare-male advantage depends upon analyzing data from the assumption that females mate randomly.

Although the studies performed to date have often found evidence for frequency-dependent selection when properly designed, its mechanism is still unknown. Therefore, further work is needed before we can adequately understand the role and extent of this selective mode in maintaining balanced polymorphisms in natural populations.

4) Amylases

Amylases are a group of digestive enzymes which split glycogen or starch into dextrin, glucose and maltose. They are widely distributed among microorganisms, invertebrates, plants and animals, and are divided into a number of classes which include endoamylases such as α-amylase, and exoamylases, such as β-amylase and the γ or glucoamylases (Thoma et al, 1970).

a) General:

Only α-amylase has been found in animals; and due to the fact that this study deals with Drosophila melanogaster it is appropriate to deal primarily with α-amylase. Amylase was discovered by Leuchs in 1831 and was originally known as " diastase ". Kuhn (1925) defined the α-amylase as a carbohydrase which gives products with an α configuration.

The most comprehensive analysis of α-amylase action on its substrates was performed by Thoma (1976, a, b) and Wakim et al (1969). The α-amylase enzyme first makes several random cuts in the long polysaccharide chain in order to produce a mixture of large and small dextrins. In the second step, repetitive hydrolysis from one end yields predominantly maltose molecules.

All animal amylases were found to exhibit maximal activity at a neutral pH; in addition, all amylases regardless of their origin behaved as typical enzymes i.e., their activity curve is bell-shaped (activity vs. pH) (Karn and Malacinki, 1978). The isoelectric point was discovered to be in the range of 5.2 to 6.4 in most animal amylases (Mayo and Carlson, 1974). Furthermore, Hoorn and Scharloo (1978) reported that amylase activity depends upon several factors including the chain length of the substrate, the pH, the temperature and the substrate concentration.

All amylases, regardless of their biological origins, contain some inorganic ion such as calcium. The removal of the latter led to a decrease in enzyme activity. However, the normal

activity of the enzyme was restored upon the readdition of the calcium ion (Vallee et al, 1959; Hsiu et al, 1964).

Purified α-amylases from a variety of animals have molecular weights in the range of 50,000 to 55,000 daltons; these values have been confirmed in a variety of techniques in several laboratories (Malacinki and Rutter, 1969; Karn and Malacinki, 1978). Further, the enzyme was shown to be monomer containing a relatively high percentage of tyrosine, tryptophan, aspartic acid and glutamic acids. Plant α-amylases, however, were found to have lower molecular weights ranging from 45,000 to 48,000 daltons, and consisted of a single polypeptide chain (Thoma et al, 1970). In contrast, bacterial amylases are reported to have molecular weights in the range of 100,000 daltons, but they are made up of several subunits (Mitchell et al, 1973).

Finally, due to the importance of amylase as being one of the major secretory products, automated methods have been developed and are presently in use in some laboratories for clinical purposes (Karn and Malacinki, 1978).

b) Drosophila Amylases:

Kikkawa and Abe (1960) were the first to lay the ground-work for the study of amylase in Drosophila melanogaster. A total of seven isoamylases or amylase patterns have been characterized by the use of the electrophoretic technique; based on their electrophoretic mobilities, these isoamylases were labelled by Kikkawa (1964) as follows: Amylase ^{1} for the fastest

anodally migrating amylase, and Amylase^{6} for the slowest one which lay closest to the origin. In addition, the Amylase^{1} pattern can be subdivided into three categories on the basis of the enzyme activity present, which are as follows: Amylase^{1-a}, Amylase^{1-b} and Amylase^{1-c}. The activity of the Amylase^{1-a} strain has approximately two times the activity of the Amylase^{1-b} strain, however, Amylase^{1-b} is twice as active as the Amylase^{1-c} strain (Kikkawa, 1964). Furthermore, Kikkawa (1964) went on to suggest that due to the fact that Amylase^{1} is the most common, it could therefore be the ancestral phenotype.

The active amylase molecular weight estimate, based on the alteration of the pore size in the polyacrylamide disc gel, has given values of roughly 50,000 daltons (Karn and Malacinki, 1978). The active molecule seems to consist of a single polypeptide chain, which is consistent with the genetic analysis. Furthermore, the optimum pH for Drosophila melanogaster α-amylase was found to be 7.4 with Tris-Hcl as a buffer (Doane, 1969b). Doane (1969b) tested the stability of Drosophila amylases by varying the temperature; it was found that the enzyme activity remained unaffected even after a full year at -20°C. Drosophila melanogaster amylases show varying degrees of specificity for different substrates, however, the highest degree of affinity was found to be for starch (Doane, 1969b).

Kikkawa and Abe (1960) pointed out that amylase activity in Drosophila melanogaster occurs mostly in the digestive tract and in the hemolymph. Doane (1969a) confirmed the above and

added that other organs and tissues also contain limited amylase activity which include the salivary glands, the muscle, the Malpighian tubules and the fat body. Further, Doane (1969b) traced the level of amylase activity during the life cycle of Drosophila melanogaster. It was found that in newly laid eggs, amylase activity is very low which persists during embryonic development, however, at the end of this stage a marked increase in activity occurs. During the larval stages, a gradual increase in amylase activity continues until the maximum is reached in the third instar larvae. Amylase activity drops during the pupal stage and remains low until the adults emerge, at which time amylase activity rises again during the first few days of adult life and reaches a plateau by the fourth day.

Furthermore, Doane (1967) examined the isozymes' activity at the tissue level and found that the relative activities of isoamylases differ in different tissues of the same organism. For example, a tissue examination of the third instar larvae of Amylase^{3,6} reveals that the Amylase^{3} enzymes are slightly more numerous than the Amylase^{6} enzymes in the haemolymph, but the reciprocal relationship is true in the midgut. Doane believes that either there is an independent regulation of the gene products within the same tissue, or that the amylase gene for isozyme three (Amylase^{3}) is regulated independently of the closely linked duplication isozyme number six (Amylase^{6}), or both. Unfortunately, the exact nature of the controlling mechanism remains unknown. Finally, the approximate cytological localization of the amylase gene indicates that it is on the right arm of the second chromosome

at 77.3 Centimorgan in Drosophila melanogaster (Kikkawa and Abe, 1960; Doane, 1969a).

(i) Genetic Regulation of Amylase Activity:

An abundant amount of information is available which touches on the genetic control of amylase in the Drosophila genus. In Drosophila melanogaster, many aspects of the genetic control and developmental regulations of the amylase enzyme, however, have already been investigated in many laboratories. Unfortunately, large gaps remain in our comprehension of the controlling steps leading from the amylase gene to the final product (Doane, 1969a).

Kikkawa & Abe (1960) suggested that a single " controlling locus " on the right arm of the second chromosome regulates amylase biosynthesis. However, hybrids from crosses between any two amylase strains always produce banding patterns which are composites of the parental amylase type with no hybrid amylase being found. This led Kikkawa (1964) to suggest that Drosophila melanogaster amylases are all " controlled by very closely linked genes ", and that the amylase loci could be allelic and co-dominant with one another.

Hoorn and Scharloo (1978) found that in the Amylase^{1} strain, amylase activity closely follows the increase in body weight, then after hatching it becomes relatively constant. On the other hand, in the Amylase^{4,6} strain, amylase activity continues to increase during adult life. These observations led Hoorn and Scharloo (1978) to suggest that the regulation of

amylase genes is quite different in the previously mentioned strains. In addition, Abraham and Doane (1978) have reported that the MAP (midgut activity pattern) locus is the regulatory gene for the α-amylase structural gene in the posterior midgut (PMG) of the adult Drosophila melanogaster. They stated that there are three allelic patterns: MAP^{A} which shows activity along most of the PMG, MAP^{B} which indicates very little activity at the anterior end of the PMG and finally MAP^{C} which has little or no activity at all. In addition they pointed out that these regulatory genes were found to be trans-acting. It has also been shown that amylase activity in heterozygotes (Amy^{6}/Amy^{1-c}) is controlled by trans-acting regulatory genes (Haj-Ahmad and Hickey, unpublished).

Finally, several reports indicated that salivary amylase in various organisms can be stimulated by various chemicals. Watson et al (1979) have shown that there is a parallelism between the calcium (Ca^{2+}) uptake and the amylase released from mouse parotid fragments. Mc Pherson and Hales (1978) reported that adrenalin stimulates the maximum release of amylase but inhibits leucine (H^{3}) incorporation in the amylase molecule. Phentolamine reverses the above effect. Cholinergic agents can stimulate amylase release, but they inhibit its biosynthesis. Insulin, however, stimulates amylase biosynthesis but not its release. Although amylase biosynthesis and release in the rat parotid seem to be closely linked, it is not yet clear whether an increase in the enzyme biosynthesis is dependent upon previous stimulation of release, or occurs via an independent mechanism

(Mc Pherson and Hales, 1978).

Lastly, the exact nature of the controlling mechanism remains to be solved, and it is of interest for the elucidation of gene regulation in eucaryotes.

(ii) Dietary Effects on Amylase Activity:

Amylase activity in Drosophila melanogaster persists in all stages from the embryo to the adult, regardless of the diet upon which the individuals are raised. However, amylase activity can be enhanced or reduced by various dietary conditicns. When flies were raised on four different diets, all of which contained the same ingredients but differed in carbohydrates, it was found that amylase activity on the starch rich diet was considerably higher than that displayed on sucrose or sucrose plus starch or glucose (Doane, 1969a). Moreover, in strains which carry duplicated amylase genes (such as, $Amy^{4,6}$) the dietary enhancement of amylase activity was not equally shared by the two major isozymes produced by this strain. For example, in adult flies raised on starch, the total activity of isozyme number three was greater than number six, even though the activity of both isozymes increased (Doane, 1969a).

Hoorn and Scharloo (1978) also studied the effect of diet on amylase activity in Drosophila melanogaster. It was found that starch intensifies amylase activity in both larvae and adults. Nevertheless, they pointed out that the ultimate effect depends greatly on other components of the food, such as the yeast

concentration. Further investigation of the effect of yeast reveals that yeast exerts the opposite effect on Amylase^{1} and Amylase^{4,6} strains, i.e., it increases amylase production in Amylase^{1} flies but lowers the enzyme activity in Amylase^{4,6} flies. However, the addition of starch to the yeast food medium has the opposite effect on amylase activity in the Amylase^{1} strain but has no result in the Amylase^{4,6} strain.

Rebound et al (1966 ,b) have shown that a rat raised on a starch rich diet synthesized almost eight times as much amylase as the rats raised on casein rich diets. Even in yeast (in Lipomyces starkeyi) Moulin and Galzy (1979) illustrated that starch induces amylase activity, but that glucose and saccharose inhibit the biosynthesis of amylase. This led Moulin and Galzy to conclude that the regulation of amylase biosynthesis depends mainly on induction by substrate.

In conclusion, it is not possible at this moment to decide by which mechanism or at which level amylase activity is influenced by diet, since there are several ways by which amylase activity can be enhanced or reduced. For example, the rate of transcription or that of translation can alter amylase activity, however, recently clear evidence supporting control at the transcription level has been documented (Hickey, personal communication).

IV MATERIALS AND METHODS

1) Genetic Stocks

Experiments were carried out using four inbred strains of *Drosophila melanogaster*, namely *Amylase* ^{null}, *Amylase* $^{1-a}$, *Amylase* ^{1}, *lobe eye* and *Amylase* ^{4,6}. *Amylase* ^{null} is an amylase deficient strain which arose as a spontaneous mutation and not as a result of genetic manipulation, which has been isolated from a wild population of flies by Hickey in 1980 (unpublished). *Amylase* $^{1-a}$ and *Amylase* ^{4,6} are amylase-producing strains and are characterized by high amylase activity. *Amylase* ^{1}, *lobe eye* is also an amylase-producing strain but is characterized by low amylase activity as compared to either *Amylase* $^{1-a}$ or *Amylase* ^{4,6}.

Flies of a given amylase genotype (*Amylase* $^{1-a}$, *Amylase* ^{4,6} and *Amylase* ^{null}) were identified either by an associated visible genetic marker (*curly wing* or *lobe eye*) or by gel electrophoresis. The wild type population of *Amylase* ^{null} and *Amylase* $^{1-a}$ have straight wings, but by classical genetic crosses with *Amylase* ^{4,6} (*curly wing*) *Amylase* ^{null} and *Amylase* $^{1-a}$ *curly wing* strains were obtained. Throughout this work the flies were maintained in 2 x 10 cm. culture vials at a constant temperature of 25 °C and at twelve hour light and darkness intervals.

2) Medium Composition

The principal compostion of one litre (1,000 ml.) of the standard food medium can be divided into four different components:

a) Killed and dried Brewer's yeast (50 g = 5%) is the source of protein, various vitamins and organic components.

b) Carbohydrates, namely soluble starch, glucose and sucrose. In the standard medium, sucrose is generally used (100 g = 10%).

c) Minerals, namely .5 g $CaCl_2$, .5 g $MgCl_2$, 8 g KNa tartarate, .5 g $FeCl_2$ and .5 g NaCl.

d) Agar (15 g = 1.5%), propionic acid (6 ml.) to prevent bacterial contamination and 950 ml. of tap water.

In the experimental medium, the two latter components (c and d) were always constant and were used in all experimental and control food media. However, the two former components (a and b) were modified. The concentration of Brewer's yeast and starch were varied by the use of factorial protocol (see Table 1).

Table 1: Combination of 3 Brewer's yeast concentrations with 3 soluble starch concentrations gives 9 different food media (A to I).

Starch Concentration	Brewer's Yeast Concentration (in %) 8	2	0.5
8%	A	B	C
2%	D	E	F
0%	G	H	I

The primary purpose of this experiment was to select the food medium which gives the greatest selective pressure against the amylase deficient strain without affecting the amylase-producing strains. The food which is poor in protein (.5% Brewer's yeast) and rich in starch (8%) seems to fit this criterion. Thus, food type C was selected and will be referred to throughout this thesis as the experimental medium. The control food medium was identical to the experimental medium except for the source of carbohydrates, the 8% starch was replaced by 8% glucose.

The survivorship of the amylase deficient ($\underline{Amy}^{null}$) and amylase-producing ($\underline{Amy}^{4,6}$) strains was tested on each of the nine different food media. When the experiment was started (t=0), the flies were six to eight days old and had been maintained during this period at 25°C on a standard food medium. Equal numbers of males and females were used in every experiment. The survivors were transferred into a fresh food medium every forty-eight hours whereas the dead flies were removed every twenty-four hours and their sex and numbers recorded. This experiment went on for ten consecutive days.

3) Densities

The survivorship of three genotypes, namely $\underline{Amylase}^{null}$, $\underline{Amylase}^{1-a}$ and $\underline{Amylase}^{4,6}$ were tested at four different densities. These densities were 10, 25, 50 and 100 flies per vial made up of 50% males and 50% females. This experiment was carried out both on the experimental and control food media. The procedures for

the density experiments are the same as those described in the monoculture (see Section 4).

The principal aim of these density tests was to select appropriate densities. The density of 100 flies per vial for the monoculture and intraspecific competition experiments was chosen for a number of reasons. Firstly, there is no significant difference in survivorship at a low density (10 flies per vial) or at a high density (100 flies per vial). In the second place, when dealing with a low density such as 10 flies per vial, the information given is much less precise. For example, if a single fly dies by experimental accident, this represents 10% of the fly population. Thirdly, and more importantly, when extreme densities are used in competition experiments, such as .1 vs. .9, this means using one single fly of a given genotype which is unacceptable.

4) Adult Monoculture

The following three genotypes were used in a monoculture experiment: $\underline{Amylase}^{null}$, $\underline{Amylase}^{1-a}$ and $\underline{Amylase}^{1}$, $\underline{lobe\ eye}$. The survivorship of the above three genotypes was monitored for twelve days on both the control and experimental food media.

The flies were aged at unknown densities for four to six days on the standard food medium at 25°C. They were then transferred and maintained at equal densities (100 flies/vial, made up of 50 males and 50 females) on a fresh standard food medium for another two days. This was done by mildly etherizing them so that sexing and counting could be performed. At time

zero, when the flies were six to eight days old, they were transferred again into the experimental and control food media.

Every twenty-four hours, the dead flies were removed from the culture medium by the use of a hair brush, and were counted and sexed under a dissecting microscope. The survivors were transferred into a fresh food medium every forty-eight hours. This procedure was followed for twelve consecutive days. A total of ten replicates were carried out for each experiment.

5) Adult Mixed Culture

The competition experiment was carried out using *Amylase*$^{1\text{-}a}$ and *Amylase*^{1}, *lobe eye* as the amylase-producing strains, and *Amylase*^{null} as the amylase deficient strain. The flies were aged in the same manner as in the monoculture. Three different frequencies were used as follows:

Table 2: Scheme for varying ratios of the genotypes being used.

Genotypes	Ratios		
	Vial 1	Vial 2	Vial 3
Amy^{null} *sw*: *Amy*^{1a} *sw*	10:90	50:50	90:10
Amy^{null} *sw*: *Amy*^{1a} *cw*	10:90	50:50	90:10
Amy^{null} *cw*: *Amy*^{1a} *sw*	10:90	50:50	90:10
Amy^{null} *sw*: *Amy*^{1}, *lobe*	10:90	50:50	90:10

where *cw* = *curly wing*; *sw* = *straight wing*; *lobe* = *lobe eyes*

Equal numbers of males and females were used in every case; the two food media used were the experimental and control food media. As in the monoculture, every twenty-four hours the dead flies were removed, and genotyped and sexed under the dissecting microscope. Furthermore, the survivors were transferred into a fresh food medium every forty-eight hours.

For genotypic identification, the curly wing and lobe eye markers, and gel electrophoresis were employed. The experiments were run for twelve consecutive days on a total of seven replicates. Replicates were done a few at a time with the materials and methods being identical in every trial. The experiments were repeated using different combinations of visible genetic markers; this controlled for " marker effects ".

6) Maltose Concentration Assay

Six to eight day old *Amylase*$^{1\text{-}a}$ and *Amylase*^{null} males were used in this experiment. 0, 10, 50, 90 and 100 males were transferred into 1.5 ml. of medium containing the same ingredients as the experimental medium except that the agar concentration was 1.0% instead of 1.5%. Only males were used in order to avoid having eggs laid in the medium, as it has been reported that eggs possess amylase activity (Doane, 1969b). Forty-eight hours later, the flies were removed and the medium was heated in boiling water for five minutes. It was then diluted with 1.5 ml. of buffer (1M of Tris, HCl, pH 7.5), and .1 ml. was assayed for maltose by the use of the DNSA assay as described by Doane (1967). Twelve

replicates were performed.

7) Amylase Enzyme Assay

a) The DNSA Assay:

The starch splitting activity of the alloenzymes was assayed by the dinitrosalicyclic acid (DNSA) method of Noelting and Bernfeld (1948) as described by Doane (1967). This method measures the number of reducing groups formed. The assay of enzyme activity was performed in a Tris-HCl buffer .1M at pH 7.4 with a .5% starch concentration.

In order to determine whether or not the flies excreted amylase into the medium, twenty Amylase $^{1-a}$ males aged six to eight days were transferred into a standard food medium twenty-four hours prior to the experiment. At the start of the experiment (t=0), these flies were transferred into three different food media:

a) 5% Brewer's yeast, 8% starch

b) .5% Brewer's yeast, 8% starch

c) 5% Brewer's yeast, 8% sucrose

Food media a and b were the experimental media whereas c was the control for the flies' ages. The flies were left on the medium for 0, 3, 6, 12, 24, and 48 hours; at the end of each period, the flies were collected and kept frozen at -20°C along with the medium they inhabited for a later analysis.

When all the samples were collected, the amount of

amylase activity in the medium and in the adult males which inhabited the media was determined by the use of the DNSA assay. Twelve replicates were performed for every time period.

b) Gel Electrophoresis:

Amylase genotypes were identified electrophoretically; in these experiments the following four amylase genotypes were used: $Amylase^{null}$, $Amylase^{1-a}$, $Amylase^{3}$ and $Amylase^{6}$. These four strains of Drosophila are particularly well suited for this kind of test because the variant $Amylase^{6}$ migrates slowly whereas $Amylase^{1-a}$ migrates relatively fast and the genotype $Amylase^{3}$ has an intermediate electrophoretic mobility. Approximately 100 males of each of the above genotypes were transferred into clean separate vials and left there for ten hours. At the end of this period, the flies were collected and homogenized in 0.1 M of Tris buffer, pH 7.5, and 5% sucrose, and the vials were washed with 0.400 ml. of this buffer. The flies along with the washable solution (excretion) were electrophoresed in separate packets on the same gel. Electrophoresis was run for three hours at 300 volts, then the gels were incubated in a starch solution made up of the following: 135 ml. of H_2O, 15 ml. of 1M Tris HCl buffer, pH 7.7, and 5 g of soluble starch. The mixture was boiled for five minutes and left to cool to room temperature before being used. The staining was done in I-KI solution (1M) for five minutes. Transparent enzymatic bands then showed up on a blue background.

The amount of amylase excreted by the larvae was determined both by the DNSA assay and gel electrophoresis. Twenty early third

instar larvae were transferred into a buffer solution made up of .1 M of Tris buffer, pH 7.5, either with 10% sucrose or 5% starch, and left for 0, 2, 4, 8, 16 and 24 hours in 1.5 ml. polyethylene tubes. At the end of each period, the solution in which the larvae were contained, was electrophoresed along with the larvae in separate packets on the same gel in order to see if any amylase was excreted by the larvae, and whether it came from the larvae or other sources.

8) Larval Carbohydrate Requirements

In order to determine the carbohydrate requirements of larvae the following combinations of food media were made:

Table 3: Combination of 3 Brewer's yeast concentrations with 5 sucrose concentrations gives 15 different food media (A to O).

Sucrose Concentration	Brewer's Yeast Concentration (in %) 6	3	2
8%	A	B	C
4%	D	E	F
2%	G	H	I
1%	J	K	L
0%	M	N	O

A 3 x 5 table was constructed in which the concentration of yeast and carbohydrates (sucrose was used) was varied, thus giving fifteen different food media. The viability of Amylase [1-a] and

Amylase^{null} was tested on each food medium. 100 eggs were transferred into each food medium in a monoculture; within fifteen days, the newly emerged flies were counted and recorded every forty-eight hours.

V RESULTS

Flies with high amylase activity such as *Amylase*^{4,6}, *Amylase*$^{1\text{-}a}$ and *Amylase*^{1}, *lobe eye* are better adapted to media containing starch than those flies with no, or very low, amylase activity such as the *Amylase*^{null} strain; as a result, selection favours high amylase activity genotypes in either a monoculture or in competition for food in a mixed culture. By increasing the concentration of starch and by decreasing the concentration of yeast in the medium (Table 1, p.44), a decrease in the fitness of the variants with low or no amylase activity is caused. Thus, the difference in fitness (measured in terms of survivorship) conferred by the amylase locus can successfully be amplified, and has been recorded in the following section.

1) Adult Survivorship on Nine Different Food Media

On each of the nine food media (Table 1,p.44) five replicate vials were run for each of two variants, namely *Amylase*^{4,6} and *Amylase*^{null}. In addition, on medium I, ten replicates were run for each of the following genotypes: *Amylase*$^{1\text{-}a}$, *Amylase*^{1}, *lobe eye* and *Amylase*^{null}. The percentage survivorship of each genotype on each of the nine food media was recorded in Tables 4, 5, 6 and 7. The values in each of the mentioned tables were used to plot the corresponding survivorship curves. The raw data on this section can be found in the appendix,

(pp.151-156, Tables 1 to 5C).

On the first three food media (A, B and C) which contained equal amounts of soluble starch (8%) and three different concentrations of dead Brewer's yeast (8%, 2% and 0.5% respectively), $\underline{Amylase}^{4,6}$ survivorship was the highest on medium C with 98 ± 2% at day 10 whereas on medium A it was 86 ± 5% after ten days (Table 4). This difference was significant ($P<.05$, $t = 2.415$).

In contrast, the amylase deficient strain ($\underline{Amylase}^{null}$) survivorship is the lowest on medium C with 0% survivors after ten days, and the highest on medium A with 76 ± 2% survivorship (Table 4). This difference is highly significant ($P<.001$, $t = 10.588$).

When the values in Table 4 were used to plot Figure 1, the difference in survivorship between the amylase-producing ($\underline{Amylase}^{4,6}$) and the amylase deficient strain can be clearly seen (Figures 1A, 1B and 1C). This difference increases as the concentration of Brewer's yeast decreases. For example, the percentage survivorship on medium A for $\underline{Amylase}^{null}$ (after ten days) was 76 ± 2% and for $\underline{Amylase}^{4,6}$ it was 86 ± 5% which does not constitute a significant difference; however, the survivorship for both genotypes respectively on medium B was 62 ± 4% for $\underline{Amylase}^{null}$ and 96 ± 4% for $\underline{Amylase}^{4,6}$. This difference was found to be strongly significant ($P<.001$, $t = 4.628$).

On media D, E and F which all contained 2% starch (25% of the concentrations of the previous three media) and three different concentrations of Brewer's yeast (8%, 2% and 0.5%

Table 4: Survivorship (percentage ± 1 S.E.) of an amylase-producing strain (Amy four, six) and an amylase deficient strain (Amy null) on three food media (first three from Table 1). These values were used to plot Figure 1 (A, B, C) on the following page.

Time in Days	Medium Number					
	A (8% starch + 8% B.Y.)		B (8% starch + 2% B.Y.)		C (8% starch + 0.5% B.Y.)	
	null Amy	4,6 Amy	null Amy	4,6 Amy	null Amy	4,6 Amy
0	100%	100%	100%	100%	100%	100%
2	96±2	98±2	96±2	100±0	88±4	100±0
4	92±2	98±2	82±4	98±2	44±5	100±0
6	88±2	94±4	70±6	96±4	22±2	100±0
8	80±0	88±4	64±5	96±4	6±2	100±0
10	76±2	86±5	62±4	96±4	0±0	98±2

where B.Y. = Brewer's yeast

respectively), Amylase 4,6 survivorship was high. Its survivorship on these three media, after ten days, was 80 ± 3% , 86 ± 6% and 88 ± 4% respectively (Table 5) but was not significantly lower than on the three previous media (A, B and C) for the same period of time (ten days). On the other hand, the Amylase null survivorship on media D, E and F after ten days was 72 ± 2%, 70 ± 3% and 4 ± 6% respectively (Table 5). Figure 2 shows that the difference in survivorship between Amylase 4,6 and Amylase null increases as the concentration of Brewer's yeast decreases.

On the last three food media (G, H and I) which contained

Figure 1: Average percentage of surviyorship of *Amy*^{4,6} and *Amy*^{null} in a monoculture on three food media (A, B and C); all contained 8% starch along with the following concentrations of Brewer's yeast: A 8%, B 2% and C 0.5%. It is apparent that the *Amy*^{4,6} (Δ---Δ) survivorship was not affected by the decrease in the concentration of Brewer's yeast. However, the *Amy*^{null} (o——o) survivorship decreased significantly as the concentration of Brewer's yeast decreased.

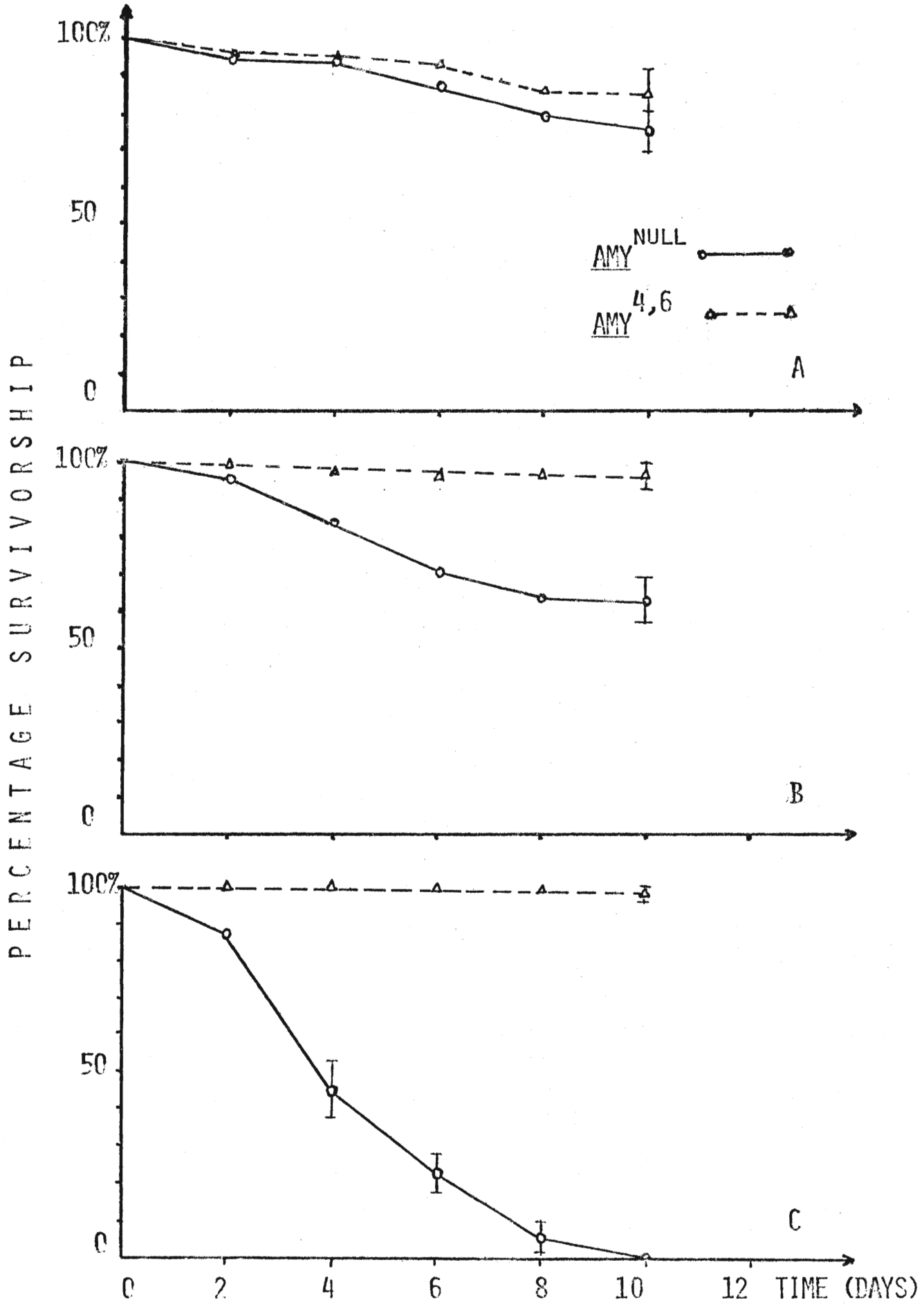

100%
50
0
AMY NULL
AMY 4,6
A
100%
50
0
B
100%
50
0
C
PERCENTAGE SURVIVORSHIP
0
2
4
6
8
10
12
TIME (DAYS)

Table 5: The mean percentage survivorship of Amy^{4,6} and Amy null on three food media which all contained 2% starch and different concentrations of Brewer's yeast (8%, 2% and 0.5%). These values were used to plot the survivorship curves (Figures 2D, 2E and 2F) on the following page.

Time in Days	Medium Number D (2% starch + 8% B.Y.)		F (2% starch + 2% B.Y.)		F (2% starch + 0.5% B.Y.)	
	null Amy	4,6 Amy	null Amy	4,6 Amy	null Amy	4,6 Amy
0	100%	100%	100%	100%	100%	100%
2	100±0	100±0	92±4	100±0	92±4	100±0
4	96±2	100±0	86±2	100±0	42±6	100±0
6	86±2	98±2	86±2	100±0	20±3	94±3
8	78±2	86±2	80±3	94±4	10±3	94±3
10	72±2	80±3	70±3	86±6	4±6	88±4

where B.Y. = Brewer's yeast

contained no carbohydrates and three different concentrations of Brewer's yeast (8%, 2% and 0.5% respectively), one would expect the survivorship of the amylase-producing and amylase deficient variants to be similar.

Table 6 and Figure 3 (G and H) show that the survivorship of both strains (Amylase^{4,6} and Amylase^{null}) on media G and H were very similar up to day 6; thenafter, a significant difference between the survivorship of Amylase^{4,6} and Amylase^{null} exists, with Amylase^{4,6} surviving better. For example, at day 10, Amylase^{null} survivorship was 66 ± 2.45% and Amylase^{4,6} percentage survivorship was 86 ± 5 % ($P < .05$, $t = 2.390$).

Figure 2: The mean percentage of survivorship of *Amy*^{4,6} and *Amy*^{null} in a monoculture on three food media D, E and F; all contained the same concentration of soluble starch (2%), but were made up of the following concentrations of Brewer's yeast: D 8%, E 2% and F 0.5%. As in the previous figure, only the *Amy*^{null} (o——o) survivorship significantly decreased as the concentration of Brewer's yeast decreased.

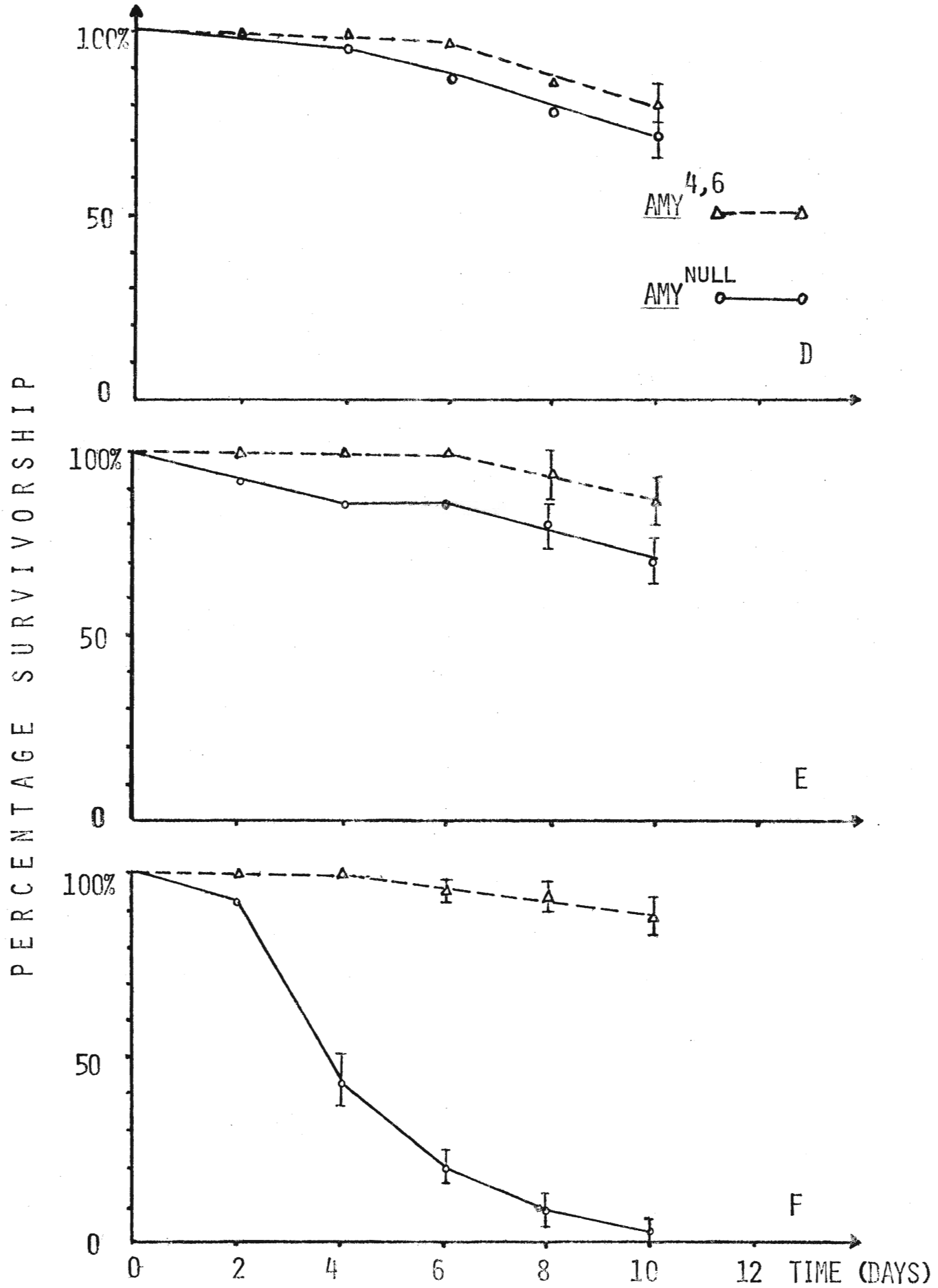

100%
50
0
AMY 4,6
AMY NULL
D
100%
50
0
E
100%
50
0
F
PERCENTAGE SURVIVORSHIP
0
2
4
6
8
10
12
TIME (DAYS)

Table 6: The mean percentage survivorship of $\underline{Amy}^{4,6}$ and $\underline{Amy}^{null}$ in a monoculture, on two media having no carbohydrates, but medium G having 8% Brewer's yeast and medium H having 2% Brewer's yeast.

Time in Days	Medium Number: G = 0% starch + 8% B. yeast		H = 0% starch + 2% B. yeast	
	$\underline{Amy}^{null}$	$\underline{Amy}^{4,6}$	$\underline{Amy}^{null}$	$\underline{Amy}^{4,6}$
0	100%	100%	100%	100%
2	98±2	100±0	98±2	100±0
4	94±2	100±0	90±3	100±0
6	80±3	96±4	82±2	100±0
8	72±4	84±2	74±2	90±3
10	52±4	78±5	66±2	86±5

where B. yeast = Brewer's yeast

Table 7 and Figure 3 (I) represent the mean percentage survivorship for $\underline{Amylase}^{null}$, $\underline{Amylase}^{1}$, $\underline{lobe\ eye}$ and $\underline{Amylase}^{1-a}$ on medium I which contains no carbohydrates and 0.5% Brewer's yeast. It can be seen that no difference exists between the amylase-producing ($\underline{Amylase}^{1}$, $\underline{lobe\ eye}$ and $\underline{Amylase}^{1-a}$) and the amylase deficient ($\underline{Amylase}^{null}$) strain survivorship on this medium which one would expect.

After analyzing the experimental results on the nine food media, one can safely conclude that in a pure culture no overall differences were found between the amylase-producing and the amylase deficient strains on media rich in Brewer's yeast where both strains survived very well (Figures 1A, 2A and 3A) and on a

Table 7: The mean percentage survivorship of three amylase variants, namely Amy null, Amy one, lobe eye and Amy one-a on a medium containing no carbohydrates.

Time in Days	Medium I (0% starch + 0.5% Brewer's yeast)		
	null Amy	1 Amy, lobe eye	1-a Amy
	100% ± 1 S.E.	100% ± 1 S.E.	100% ± 1 S.E.
0			
2	81 ±0	77 ±2	93 ±1
4	47 ±1	35 ±2	60 ±2
6	6 ±1	7 ±1	24 ±1
8	1 ±0	1 ±1	8 ±1
10	0	1 ±0	2 ±1
12	0	0	±0

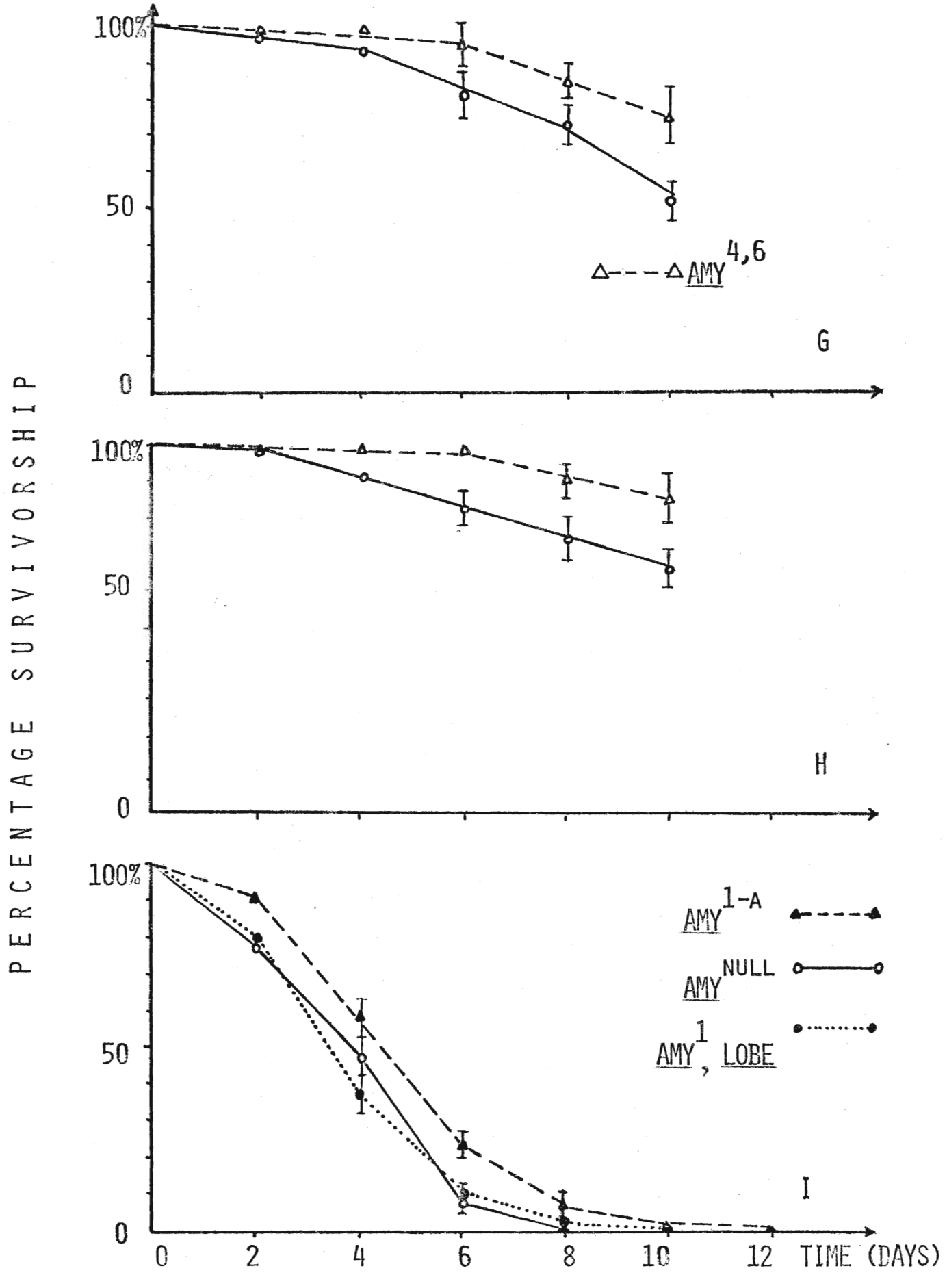
where 1 S.E. = 1 standard of error

medium containing no carbohydrates and 0.5% Brewer's yeast where both strains died in a similar manner (Table 7). Furthermore, on media C and F there was a significant difference between the survivorship of the amylase-producing and amylase deficient strains (Figures 1C and 2F). This difference in survivorship can be attributed to the amylase locus. Thus, the Brewer's yeast concentration determines what effect the ability to utilize starch will have on the flies' survival. In other words, at a low concentration of Brewer's yeast (0.5%) the flies will survive if they can utilize the starch, and they will not survive if they fail to utilize starch as a source of carbohydrates.

Since medium C gives the highest selective pressure against Amylase null without affecting an amylase-producing strain,

Figure 3 (G and H): Percentage of survivorship curves for $Amy^{4,6}$ and Amy^{null} on media G and H. Both media contained no carbohydrates but contained 8% and 2% of Brewer's yeast respectively. These results are the average of five replicates, each replicate being made up of five males and five females.

Figure 3 (I): Mean survivorship curve for three amylase variants, namely Amy^{null}, Amy^{1}, lobe eye and Amy^{1-a} in a monoculture on medium I which contained no carbohydrates and a low concentration of Brewer's yeast (0.5%). These results represent the mean of ten replicates; each genotypic replicate was made up of 100 flies, 50 males and 50 females. It can be clearly seen that the Amy^{1-a} (▲), Amy^{1}, lobe eye (●) and Amy^{null} (O) percentage survivorships were very similar as one would predict, since the possession of amylase activity does not confer any fitness on this medium.


PERCENTAGE SURVIVORSHIP
100%
50
0
AMY 4,6
G
100%
50
0
H
100%
50
0
AMY 1-A
AMY NULL
AMY 1, LOBE
I
0
2
4
6
8
10
12
TIME (DAYS)

it was chosen as the experimental medium for further experiments.

2) Amylase^{4,6}, Amylase^{1-a} and Amylase^{null} Survivorship at Various Densities

The effect of initial density on survivorship was tested on medium C which contained 8% starch and 0.5% Brewer's yeast, and on the control medium which is identical to medium C except that 8% glucose replaced the 8% starch. The raw data for this section can be found in the appendix,(pp. 157, Table 6.).

In Table 8, the results for Amylase^{null} survivorship on the starch and glucose food media are given. It is apparent that Amylase^{null} survivorship at all densities was very high on the glucose food medium (control); on the other hand, on the starch food medium (experimental medium) its survivorship reached 0% within eight days, regardless of the initial densities (whether 100, 50, 25 or 10 flies per vial).

In contrast to the Amylase^{null} survivorship which is 0% at day 10 on the starch food medium at any of the four densities, the Amylase^{1-a} survivorship was over 90% for all densities (Table 9); whereas the Amylase^{4,6} survivorship was over 80% at three of the four densities (Table 10). At the fourth density (10 flies per vial) the survivorship of Amylase^{4,6} was 70%, but it is still significantly different from 0% survivorship for Amylase^{null} on the same type of medium and at the same density.

On glucose (control) food medium, the survivorship was

Table 8: Percentage survivorship of *Amy*^{null} at four different densities (100, 50, 25 and 10 flies/vial, made up of 50% males and 50% females) and on two different media (8% starch and 8% glucose).

Time in Days	Experimental Medium				Control Medium			
	Densities = Flies/vial				Densities = Flies/vial			
	100	50	25	10	100	50	25	10
0	100%	100%	100%	100%	100%	100%	100%	100%
2	82	90	92	100	90	100	100	100
4	30	24	24	50	90	98	100	100
6	4	2	4	10	89	96	100	90
8	0	0	0	0	88	96	96	90
10	0	0	0	0	87	94	96	90

Table 9: Percentage survivorship of *Amy*$^{1\text{-}a}$ at four densities and on two different media.

Time in Days	Experimental Medium (8%s)				Control Medium (8%g)			
	Densities = Flies/vial				Densities = Flies/vial			
	100	50	25	10	100	50	25	10
0	100%	100%	100%	100%	100%	100%	100%	100%
2	100	98	100	100	99	100	100	100
4	100	98	100	100	93	92	96	90
6	99	98	100	100	92	90	92	80
8	98	98	96	100	88	86	88	80
10	96	96	96	100	87	84	84	70

where 8%s = 8% starch, and 8%g = 8% glucose.

Table 10: Percentage survivorship of Amy^{4,6} at four densities and on two different media.

Time in Days	Experimental Medium: Densities = Flies/vial 100	50	25	10	Control Medium: Densities = Flies/vial 100	50	25	10
0	100%	100%	100%	100%	100%	100%	100%	100%
2	99	100	100	100	96	100	96	100
4	93	92	96	90	90	94	96	100
6	92	90	92	80	88	90	96	100
8	88	86	88	80	88	88	92	90
10	87	84	84	70	88	88	92	90

well over 80% for all three genotypes; in other words, no difference in survivorship can be found on the glucose food medium.

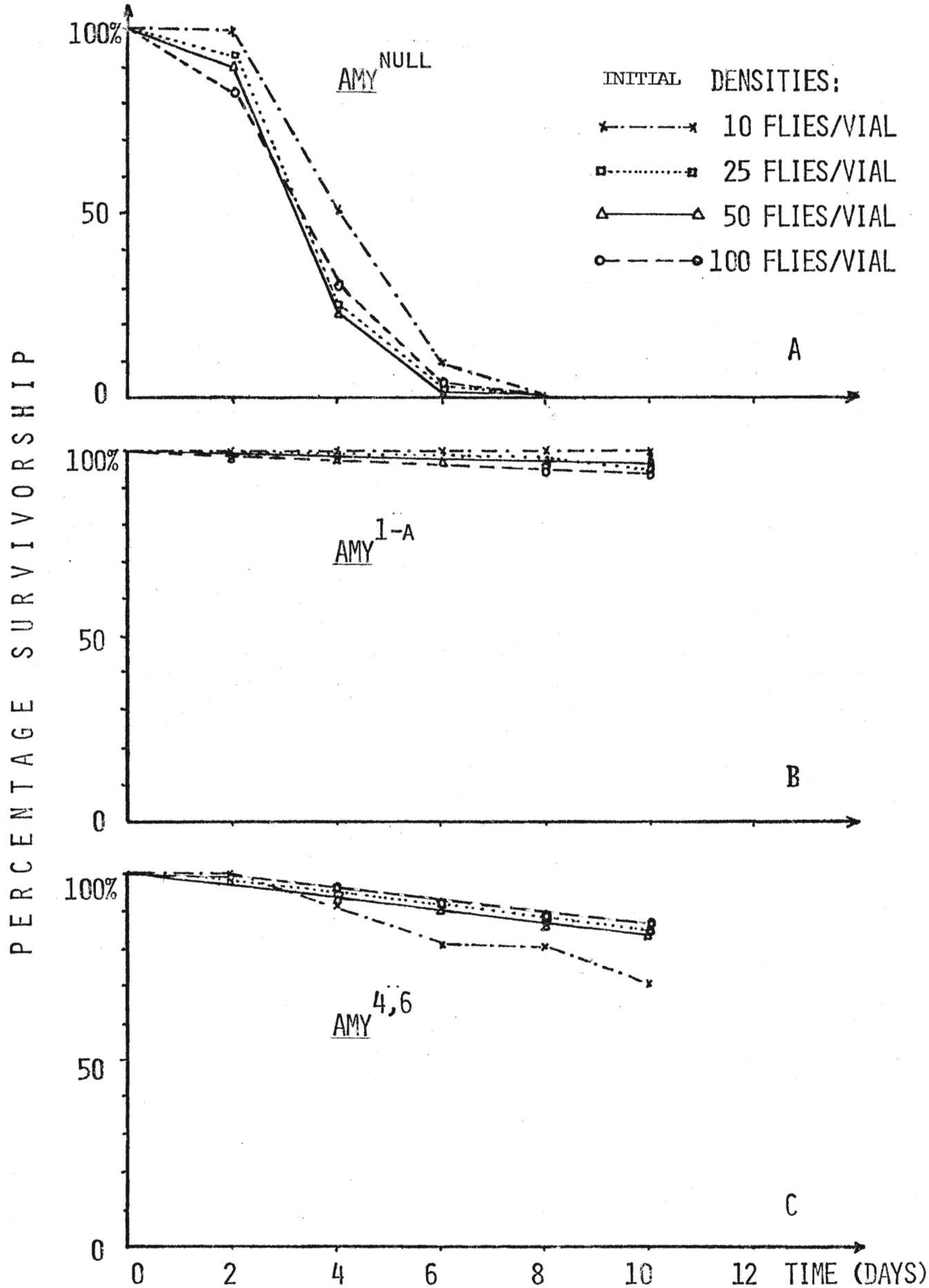
Translating the survivorship values on starch for Amylase^{null}, Amylase^{1-a} and Amylase^{4,6} (from Tables 8, 9 and 10) into survivorship curves (Figures 4A, 4B and 4C respectively), help to demonstrate the differential survivorship between the amylase deficient (Amylase^{null}) and the amylase-producing (Amylase^{1-a} and Amylase^{4,6}) strains. It is clear that Amylase^{null} survivorship reached 0% at day 8 (Figure 4A) for all densities whereas Amylase^{1-a} (Figure 4B) and Amylase^{4,6} (Figure 4C) survivorship curves at day 8 were well over 80% for all densities.

It must be concluded at this stage that in a pure culture, no difference was found between the survivorships within a strain that could be attributed to the different densities being used.

Figure 4A: Percentage survivorship of $\underline{Amy}^{null}$ on the experimental medium which contains 8% starch and 0.5% Brewer's yeast, at four different densities (100, 50, 25 and 10 flies per vial of which 50% are males and 50% females). It is apparent that the overall outcome of $\underline{Amy}^{null}$ survivorship was not affected by the densities being used.

Figure 4B: Percentage survivorship of $\underline{Amy}^{1-a}$ on the experimental medium at four different densities. Note again that survivorship does not seem to be affected by these densities.

Figure 4C: Percentage survivorship curves for $\underline{Amy}^{4,6}$ on the experimental medium at the four densities mentioned above.


100%
50
0
AMY NULL
INITIAL DENSITIES:
10 FLIES/VIAL
25 FLIES/VIAL
50 FLIES/VIAL
100 FLIES/VIAL
A
100%
50
0
AMY 1-A
B
100%
50
0
AMY 4,6
C
PERCENTAGE SURVIVORSHIP
0
2
4
6
8
10
12
TIME (DAYS)

3) Monoculture of Amylase^{1-a}, Amylase^{1}, lobe eye and Amylase^{null} on Starch and Glucose Food Media

After having selected medium C which contains 0.5% Brewer's yeast and 8% starch, as the medium which gives the desired selective pressure on the amylase deficient strain (Amylase^{null}) without affecting the survivorship of the amylase-producing strains (Amylase^{1-a}, Amylase^{1}, lobe eye), the density of 100 flies per vial was also selected as the appropriate density. The raw data for the monoculture experiments can be found in the appendix,(pp.158-168, Tables 7A to 11B.).

On each of the starch and glucose food media, ten replicate vials were run for each genotype (Amylase^{1-a}, Amylase^{1}, lobe eye and Amylase^{null}). The results on the survivorship of Amylase^{1-a} in association with straight wing (Table 11A and Figure 5A) and curly wing (Table 11B and Figure 5B) visible genetic markers point out firstly that no significant difference was found between male or female survivorship regardless of the visible genetic marker or the media they were raised on; and secondly, that the Amylase^{1-a} survivorship was very high on both the starch and glucose food media. Figures 5A and 5B help to illustrate the above statement.

Table 11A: The mean percentage survivorship of *Amy* 1-a straight wing on two media for males and females. Male and female survivorship values were averaged in order to plot the *Amy* one-A straight wing survivorship curve (Figure 5A).

Time in Days	*Amy* 1-a straight wing ♂♂	*Amy* 1-a straight wing ♀♀	Stat. sign.
	% survivors ± 1 SEM	% survivors ± 1 SEM	
	Experimental Medium: 8% starch + 0.5% B. yeast		
0	100 ±0	100 ±0	NS**
2	98 ±1	99 ±0	NS
4	95 ±1	98 ±1	NS
6	93 ±2	96 ±1	NS
8	92 ±2	96 ±1	NS
10	89 ±3	94 ±2	NS
12	87 ±3	92 ±2	NS
	Control Medium: 8% glucose; 0.5% B. yeast		
0	100 ±0	100 ±0	NS
2	100 ±0	99 ±0	NS
4	100 ±0	99 ±0	NS
6	99 ±1	99 ±0	NS
8	98 ±1	98 ±1	NS
10	97 ±1	97 ±1	NS
12	95 ±1	96 ±1	NS

where % survivors ± 1 SEM = % survivors ± 1 standard deviation of the mean

** NS = not significant ($P \geq .05$)

Table 11B: The mean percentage of survivorship of Amy 1-a curly wing on two media for males and females. The survivorship values of the males and females were averaged in order to plot the Amy 1-a curly wing survivorship curve (Figure 5B).

Time in Days	Amy 1-a curly wing ♂♂	Amy 1-a curly wing ♀♀	Stat. sign.
	% survivors ± 1 SEM	% survivors ± 1 SEM	
	Experimental Medium: 8% starch + 0.5% B. yeast		
0	100 ±0	100 ±0	NS**
2	99 ±1	99 ±0	NS
4	98 ±1	99 ±0	NS
6	97 ±1	98 ±0	NS
8	95 ±1	97 ±0	NS
10	94. ±1	97 ±1	NS
12	93. ±2	97 ±1	NS
	Control Medium: 8% glucose; 0.5% B. yeast		
0	100 ±0	100 ±0	NS
2	99 ±0	100 ±0	NS
4	99 ±0	99 ±0	NS
6	98 ±1	99 ±0	NS
8	98 ±1	98 ±1	NS
10	97 ±1	98 ±1	NS
12	96 ±1	97 ±1	NS

where % survivors ± 1 SEM = % survivors ± 1 standard deviation of the mean

** NS = not significant

Figure 5A: Survivorship curves of $\underline{Amy}^{1-a}$ straight wing on starch (O) and on glucose (Δ) food media. It can be seen that the $\underline{Amy}^{1-a}$ survivorship (whether straight wing Figure 5A, or curly wing Figure 5B) was very similar on the starch (o) and glucose (Δ) food media. In addition, reversion of the visible curly wing marker did not affect the $\underline{Amy}^{1-a}$ survivorship.

Figure 5B: Survivorship curves of $\underline{Amy}^{1-a}$ curly wing on starch (O) and glucose (Δ) food media.

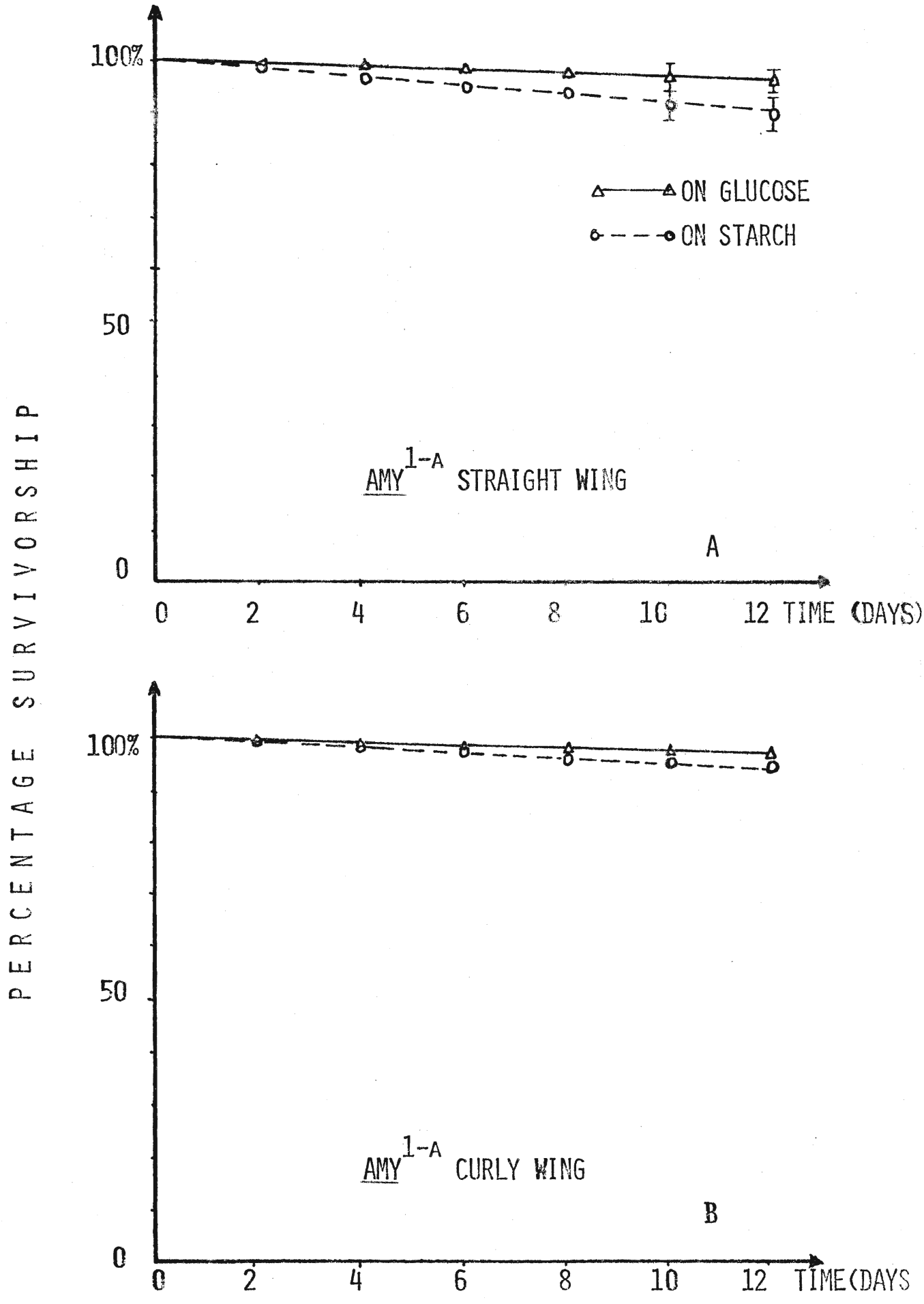

PERCENTAGE SURVIVORSHIP
100%
50
0
ON GLUCOSE
ON STARCH
AMY 1-A STRAIGHT WING
A
0
2
4
6
8
10
12 TIME (DAYS)
100%
50
0
AMY 1-A CURLY WING
B
0
2
4
6
8
10
12 TIME(DAYS

Table 12 contains the average percentage survivorship of ^{1}*Amylase* , *lobe eye* on the starch and glucose food media. Let us first compare the male to female survivorship. Although female survivorship was generally higher than that of the males at any given time, this difference was found to be insignificant. Now let us focus on the ^{1}*Amylase* , *lobe eye* survivorship on starch and glucose which is clearly illustrated in Figure 6. It is apparent that its survivorship on starch is lower than on glucose for any given period of time; nevertheless, this difference only becomes significant at day 10 and thereafter.

Table 13A contains the mean percentage survivorship of ^{null}*Amylase* *curly wing* on the starch and glucose food media. First, let us compare the survivorship of the males and females. On starch as well as on glucose, the females survived better than the males, however, this difference was only significant on day 4 on the starch food medium where the male survivorship was 5 $\pm$ 1% whereas the female survivorship was 34 $\pm$ 4% (Table 13A). Figure 7A clearly illustrates these statements.

Altering the visible genetic marker did not change the outcome of the experiment. It can be seen (Table 13B) that very similar results were obtained on the ^{null}*Amylase* *straight wing* strain. A comparison of Figures 7A to 7B shows the extent of the similarity between the survivorship of ^{null}*Amylase* *curly wing*

Table 12; The percentage survivorship of *Amy*^{1} *lobe eye* on two media (experimental medium and control medium) for males and females. Male and female survivorship values were averaged in order to plot the *Amy* one, *lobe eye* survivorship curve (Figure 6).

Time in Days	*Amy*^{1}, *lobe eye* ♂♂	*Amy*^{1}, *lobe eye* ♀♀	Stat. sign.
	% survivors ± 1 SEM	% survivors ± 1 SEM	
	Experimental Medium: 8% starch + 0.5% B. yeast		
0	100 ±0	100 ±0	NS**
2	96 ±2	98 ±1	NS
4	91 ±2	96 ±1	NS
6	88 ±4	94 ±2	NS
8	86 ±4	92 ±2	NS
10	82 ±5	90 ±2	NS
12	78 ±6	87 ±3	NS
	Control Medium: 8% glucose; 0.5% B. yeast		
0	100 ±0	100 ±0	NS
2	99 ±0	100 ±0	NS
4	98 ±1	99 ±0	NS
6	98 ±1	98 ±0	NS
8	97 ±1	98 ±0	NS
10	97 ±1	98 ±0	NS
12	96 ±1	97 ±0	NS

where % survivors ± 1 SEM = % survivors ± 1 standard deviation of the mean

** NS = not significant

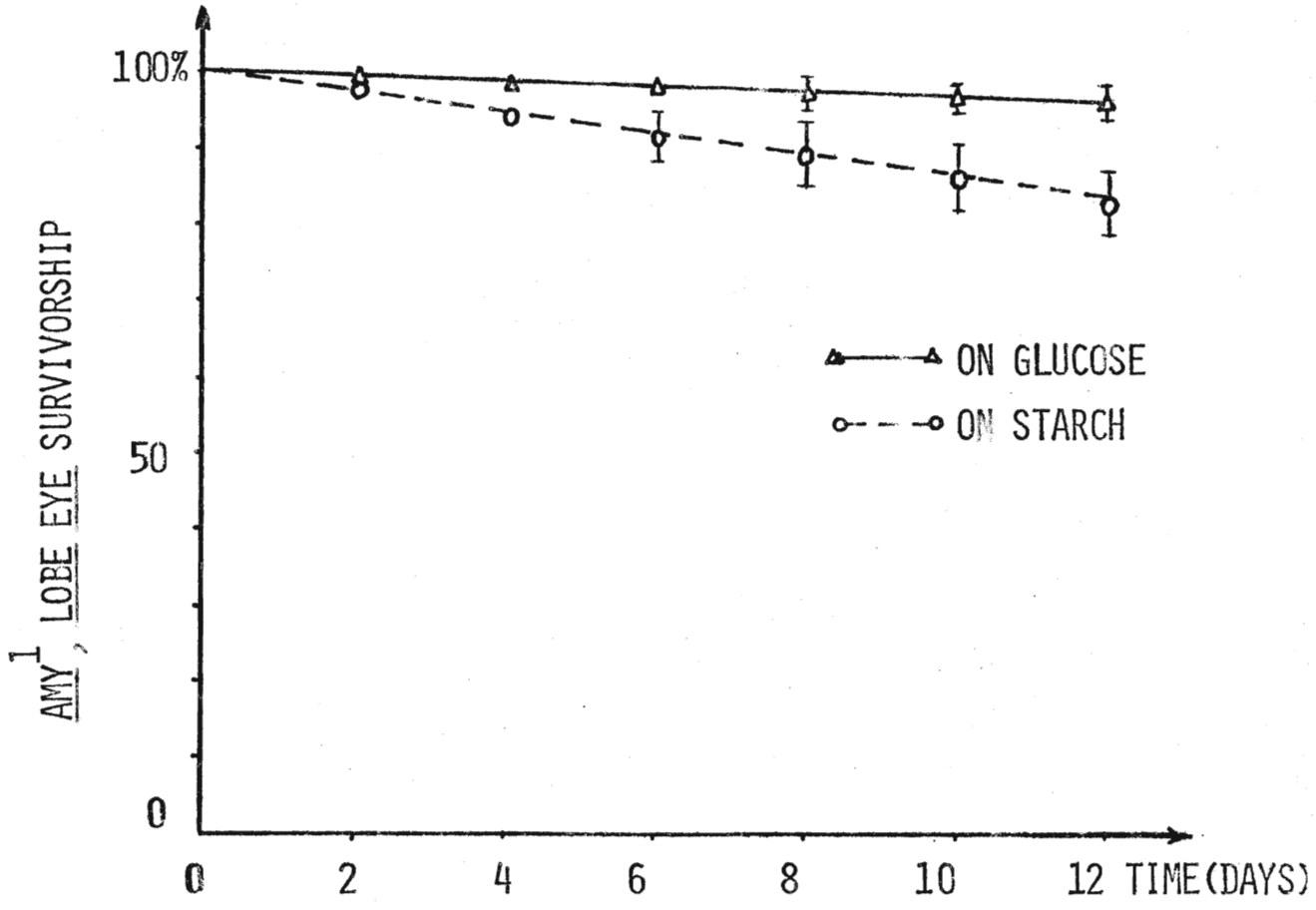


Figure 6: Survivorship of $\underline{\text{Amy}}^{1}$, lobe eye (in percentages) on two media, both poor in protein (.5% Brewer's yeast) but one is rich in starch (o- - -o) and the second is rich in glucose (Δ———Δ). It is clear that $\underline{\text{Amy}}^{1}$, lobe eye survivorship on starch was lower than its survivorship on glucose, although this difference was not significant.

Table 13A: The mean percentage of survivorship of null Amy curly wing on two media. The values in this table and in Table 10B were used to plot the survivorship curves for males and females in Figures 7A and 7B respectively.

Time in Days	null Amy curly wing ♂♂	null Amy curly wing ♀♀	Stat. sign.
	Experimental Medium: 8% starch + 0.5% B. yeast		
	% survivors ± 1 SEM	% survivors ± 1 SEM	
0	100 ±0	100 ±0	NS*
2	91 ±5	97 ±2	NS
4	5 ±1	34 ±4	SD**
6	-	3 ±1	-
8	-	-	-
10	-	-	-
12	-	-	-
	Control Medium: 8% glucose; 0.5% B. yeast		
0	100 ±0	100 ±0	NS
2	99 ±0	100 ±0	NS
4	98 ±0	99 ±0	NS
6	97 ±1	99 ±0	NS
8	97 ±1	98 ±1	NS
10	95 ±1	97 ±1	NS
12	95 ±1	97 ±1	NS

where % survivors ± 1 SEM = % survivors + 1 standard deviation of the mean

* NS = not significant

** SD = significant difference ($P < .001$, $t = 5.565$)

Table 13B: The mean percentage survivorship of *Amy*^{null} *straight wing* on two media.

Time in Days	null *Amy* *straight wing* ♂♂	null *Amy* *straight wing* ♀♀	Stat. sign.
	Experimental Medium: 8% starch + 0.5% B. yeast		
	% survivors ± 1 SEM	% survivors ± 1 SEM	
0	100 ±0	100 ±0	NS*
2	94 ±2	97 ±2	NS
4	8 ±2	60 ±5	SD**
6	-	5 ±1	SD
8	-	-	-
10	-	-	-
12	-	-	-
	Control Medium: 8% glucose; 0.5% B. yeast		
0	100 ±0	100 ±0	NS
2	100 ±0	100 ±0	NS
4	99 ±0	100 ±1	NS
6	98 ±1	99 ±1	NS
8	97 ±2	99 ±1	NS
10	97 ±2	99 ±1	NS
12	96 ±2	98 ±1	NS

where % survivors ± 1 SEM = % survivors + 1 standard deviation of the mean

* NS = not significant

** SD = significant difference ($P < .001$, $t = 8.128$)

Figure 7A: Amylase^{null} curly wing survivorship curve representing males and females on two media (experimental medium (starch) and control medium (glucose). It is self-explanatory that both male and female (whether curly wing Figure 7A, or straight wing Figure 7B) survivorship was significantly higher on glucose than on the experimental medium.

Figure 7B: Amylase^{null} straight wing survivorship curve for males and females on two media. Females (whether straight wing or curly wing) survived better than males on the starch food medium.

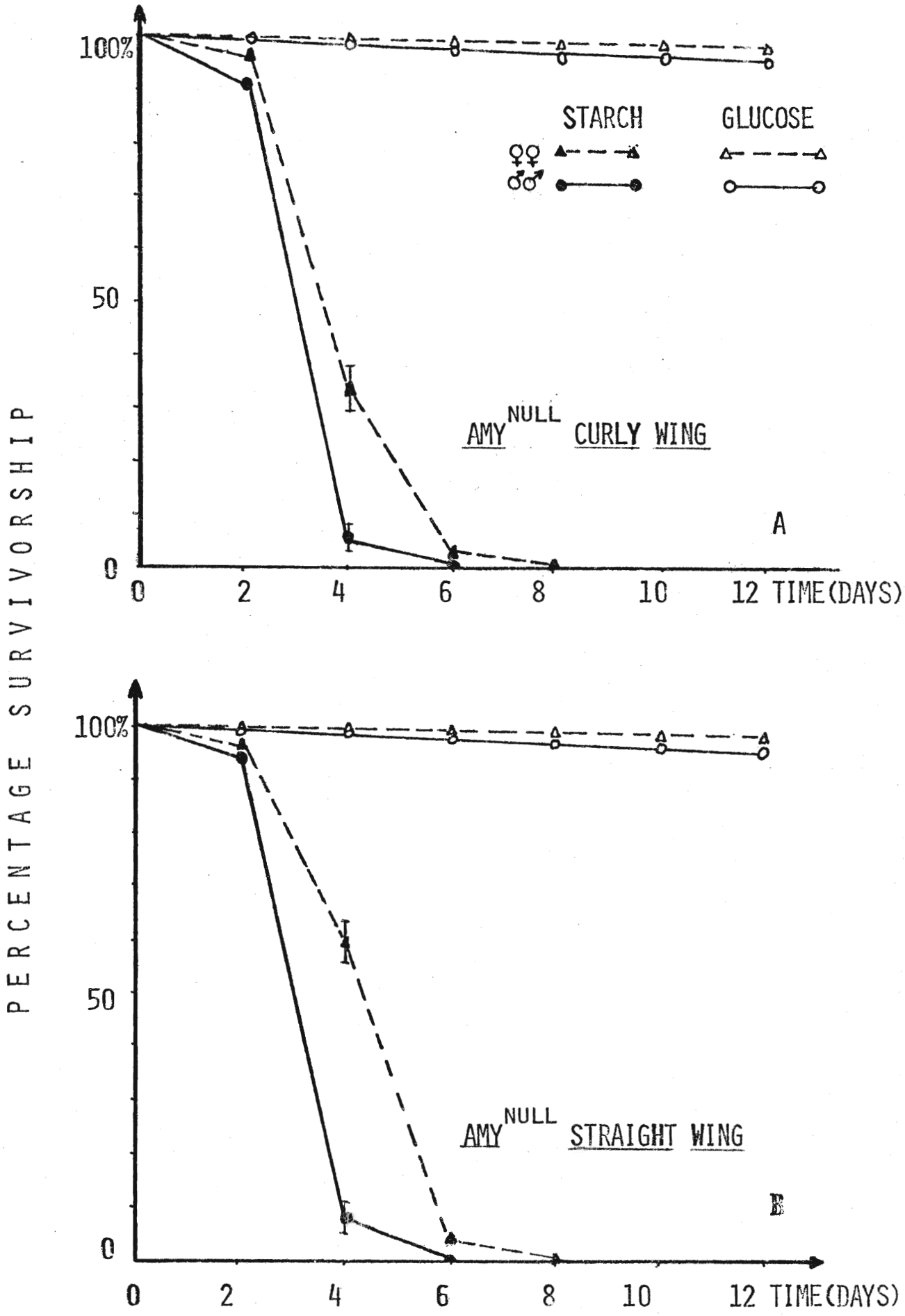
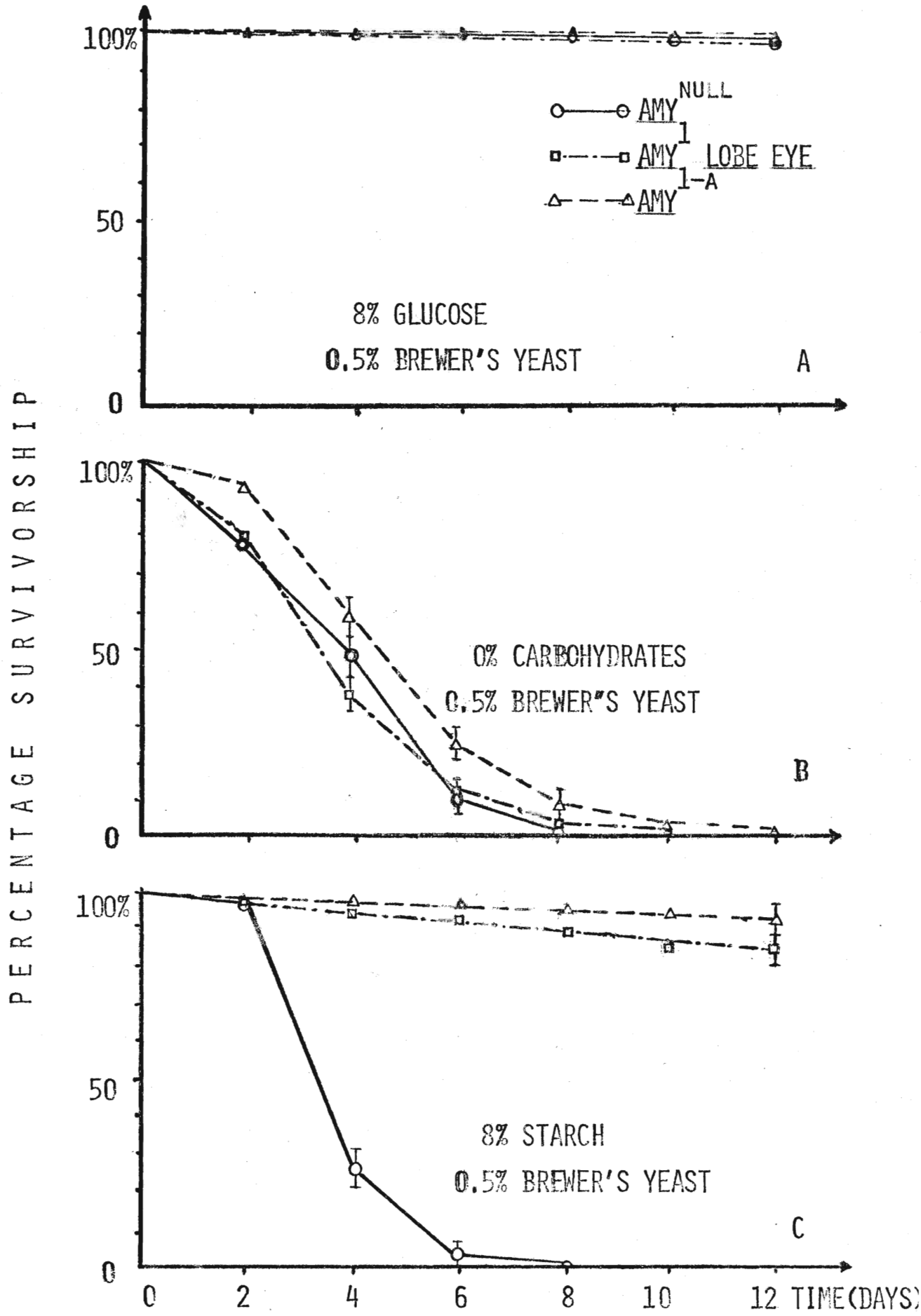

PERCENTAGE SURVIVORSHIP
100%
50
0
STARCH
GLUCOSE
♀♀
♂♂
AMY^NULL CURLY WING
A
0
2
4
6
8
10
12 TIME(DAYS)
100%
50
0
AMY^NULL STRAIGHT WING
B
0
2
4
6
8
10
12 TIME(DAYS)

Figure 8A: The percentage survivorship of three amylase variants, namely Amy^{1-a}, Amy^{1}, $lobe^{eye}$ and Amy^{null} on the control medium (0.5% Brewer's yeast and 8% glucose).

Figure 8B: The survivorship of the same amylase variants on a medium having a low amount of Brewer's yeast (0.5%) and no carbohydrates. On this medium the survivorship of the three genotypes is almost the same and approached 0% within eight days.

Figure 8C: The survivorship of the same amylase variants on the experimental medium (0.5% Brewer's yeast and 8% starch).


100%
50
0
AMY NULL
AMY 1
AMY 1-A
LOBE EYE
8% GLUCOSE
0.5% BREWER'S YEAST
A
PERCENTAGE SURVIVORSHIP
100%
50
0
0% CARBOHYDRATES
0.5% BREWER'S YEAST
B
100%
50
0
8% STARCH
0.5% BREWER'S YEAST
C
0
2
4
6
8
10
12 TIME(DAYS)

and Amylase^{null} straight wing.

Figures 8A, 8B and 8C are summary figures showing that on the glucose food medium (Figure 8A) the survivorship of the three genotypes are almost identical and close to 100%; whereas on the medium having 0.5% Brewer's yeast and no carbohydrates, the survivorship of the three genotypes are again almost the same but approached 0% within eight days (Figure 8B). In summary, supplementing the medium with 8% starch significantly improved the amylase-producing strains' survivorship (Amylase^{1-a} and Amylase^{1}, lobe eye) but it did not improve the survivorship of the amylase deficient strain (Amylase^{null}) (Figure 8C).

4) Intraspecific Competition on Starch and Glucose Food Media

This section will deal with the outcome of intraspecific competition on two media, namely starch (medium C) and glucose (control medium).

Competition experiments were carried out using Amylase^{null} with either Amylase^{1-a} or with Amylase^{1}, lobe eye. Since all strains survived well on glucose (the control medium) regardless of the frequency used, only one table (Table 14B) will be presented on this in the results section; nevertheless, data are available on these experiments in the appendix,(pp.169-190, Tables 12A to 23B, the bottom half of every table). In addition, the survivorship of the amylase-producing strains (Amylase^{1-a} and Amylase^{1}, lobe eye) on starch was also high just as in the

monoculture, therefore data on Amylase^{1-a} and Amylase^{1}, lobe eye are presented in the appendix, (pp.169-190, Tables 12A to 23B, the upper half of each table). Only the survivorship of Amylase^{null} changed significantly in these experiments. This change was dependent on its frequency. This section presents the data on the survivorship of Amylase^{null} at four frequencies (.1, .5, .9 and 1); a t-test was used to compare the survivorship at different frequencies; the t-test was performed on the transformed percentages by the use of the Arcsin transformation table.

Table 14A contains the results on Amylase^{null} straight wing when competing with Amylase^{1-a} curly wing on the starch food medium. It is clear that the Amylase^{null} survivorship improved significantly when its frequency decreased. For example, when its frequency decreased from 1 to .9 to .5 to .1, its survivorship increased from 0% to 12 $\pm$ 3% to 86 $\pm$ 3% to 95 $\pm$ 3% respectively at day 10 (Table 14A). Such observed frequency-dependent survivorship can be seen not only at day 10 but at any given time of the experiment. The transformed percentages on which a t-test was performed can be found in the appendix, (pp.191-194, in Tables 24 to 27.)

Table 14B represents the mean percentage survivorship of Amylase^{null} straight wing when competing with Amylase^{1-a} curly

Table 14A: The mean percentage survivorship of Amy^{null} straight wing when competing with Amy^{1-a} curly wing at three different frequencies (.1, .5 and .9) on the starch food medium (medium C). These values were used to plot Figure 9.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast											
	Amy^{null} (straight wing) Frequency								Statistical Comparison			
	.1		.5		.9		1.0		.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	100	±0	100	±0	100	±0	100	±0	NS	NS	NS	NS
2	100	±0	100	±0	97	±2	96	±2	NS	NS	NS	NS
4	100	±0	99	±1	66	±4	34	±3	NS	***	***	***
6	100	±0	93	±2	31	±5	2	±1	**	***	***	***
8	95	±3	88	±2	17	±4		-	NS	***	***	***
10	95	±3	86	±3	12	±3		-	*	***	***	***
12	84	±7	71	±3	8	±2		-	**	***	***	***

Transformed percentages by the arcsin transformation are in the appendix,(pp. 191, Table 24.).
NS = not significant, $P \geq 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 14B: The mean percentage of survivorship of *Amy*^{null} *straight wing* when competing with *Amy*$^{1-a}$ *curly wing* at three frequencies on the glucose (control) food medium. It can be clearly seen in this table that *Amy*^{null} survivorship was almost identical at any of the four frequencies used on this medium.

Time in Days	Control Medium: 8% glucose; 0.5% Brewer's yeast											
	Amy^{null} (*straight wing*) Frequency								Statistical Comparison			
	.1		.5		.9		1.0		.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	100	±0	100	±0	100	±0	100	±0	NS	NS	NS	NS
2	100	±0	99	±0	100	±0	100	±0	NS	NS	NS	NS
4	100	±0	98	±1	98	±1	100	±0	NS	NS	NS	NS
6	100	±0	98	±1	97	±1	99	±1	NS	NS	NS	NS
8	100	±0	98	±1	97	±1	98	±1	NS	NS	NS	NS
10	100	±0	97	±1	96	±1	98	±1	NS	NS	NS	NS
12	99	±1	97	±1	96	±1	97	±2	NS	NS	NS	NS

where NS = not significant, $P \geq 0.05$

wing on the glucose food medium. A quick examination of Table 14B indicates that Amylase^{null} survivorship was over 95% regardless of the frequency being used.

Reversing the association with the visible curly wing genetic marker did not affect the results. Table 15 shows that when Amylase^{null} curly wing competed with Amylase^{1-a} straight wing, the Amylase^{null} curly wing survivorship is very similar to that of Amylase^{null} straight wing (Table 14A). Perhaps, comparing Figures 9 and 10 may help to illustrate the above point. Drawing a straight horizontal line at the 90% survivorship level and taking the point of intercepts for each frequency will give the LT10 (Figures 11 and 12). The LT10 is the time required till 10% of the total fly population has died. Figures 11 and 12 show that when the Amylase^{null} (whether straight wing or curly wing) frequency is high, at 1 for example, it takes only two days for 10% of the fly population to die, but when its frequency is low, .1 for example, it takes approximately ten days for 10% of the total fly population to die.

What would happen if one used no visible genetic marker, i.e., let Amylase^{null} straight wing compete with Amylase^{1-a} straight wing using electrophoresis for identification; Table 16 contains the results of such a competition. These percentages represent the average of two replicates; nevertheless, the trend did not change. In other words, the Amylase^{null} survivorship remained strongly frequency-dependent.

Table 15: The mean percentage of survivorship of Amy^{null} curly wing when competing with Amy^{1-a} straight wing at three different frequencies on the starch food medium.

These values were used to plot Figure 10.

Time in Days	Experimental Medium: 8% starch; 0.5% Brewer's yeast											
	Amy^{null} (curly wing) Frequency								Statistical Comparison			
	.1		.5		.9		1.0		.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	100	±0	100	±0	100	±0	100	±0	NS	NS	NS	NS
2	99	±1	100	±0	96	±2	94	±3	NS	*	NS	*
4	99	±1	98	±1	60	±5	20	±2	NS	***	***	***
6	98	±1	94	±2	33	±7	2	±1	NS	***	***	***
8	95	±3	86	±3	21	±5		–	*	***	***	***
10	89	±4	78	±3	14	±3		-	*	***	***	***
12	82	±5	70	±4	10	±2		-	*	***	***	***

The transformed percentages by the arcsin transformation are in the appendix,(pp. 192, Table 25.).

NS = not significant, $P \geq 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Figure 9: $\underline{Amy}^{null}$ straight wing survivorship curves at various frequencies when the latter strain is competing with $\underline{Amy}^{1-a}$ curly wing on the starch food medium; the bars are standards of error. The straight line at the 90% survivorship level indicates the LT_{10} (Figure 11).

Figure 10: The same as the above, except that the wing markers were reversed: competition between $\underline{Amy}^{null}$ curly wing and $\underline{Amy}^{1-a}$ straight wing. The straight line at the 90% survivorship level indicates the LT_{10} (Figure 12).

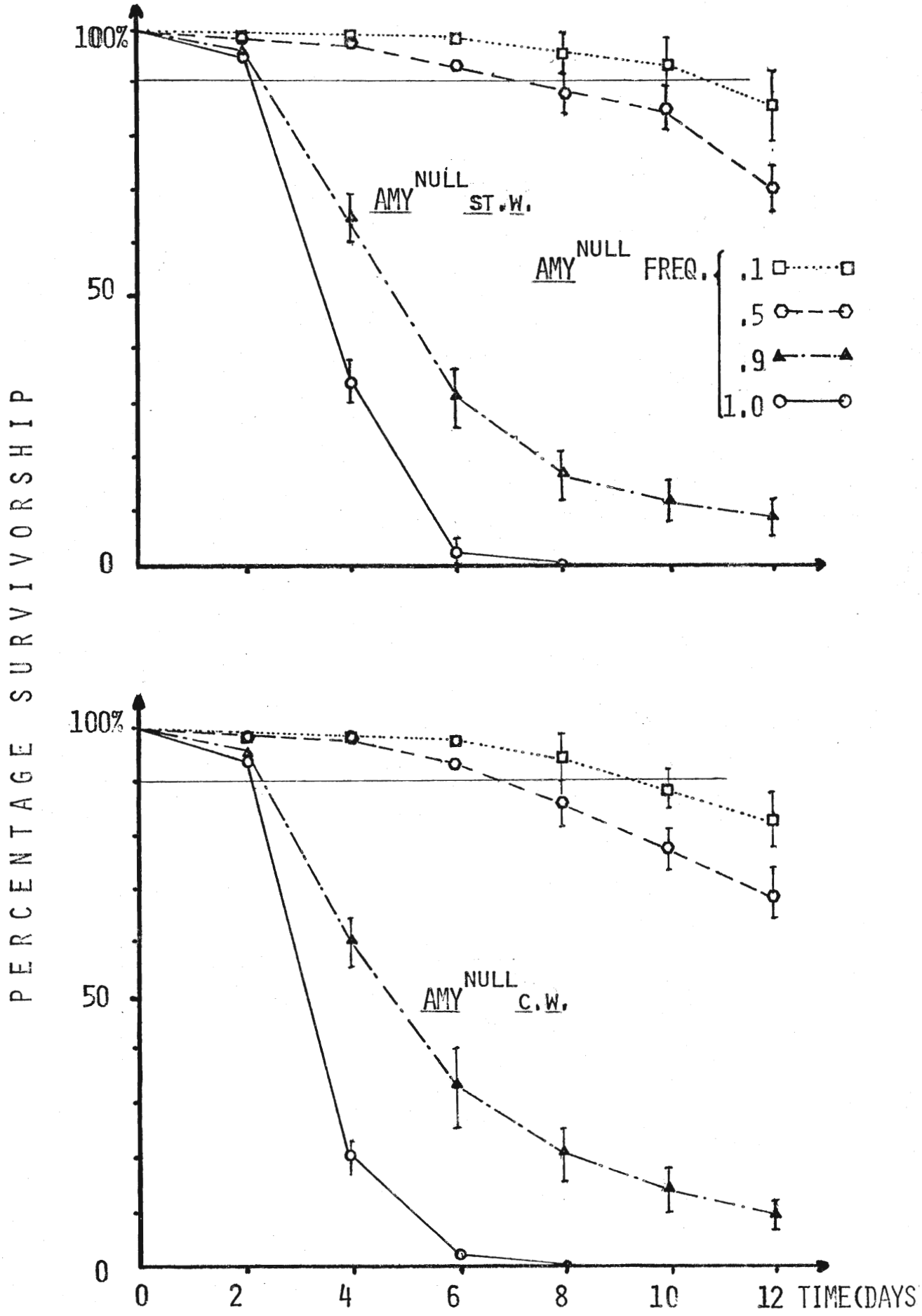
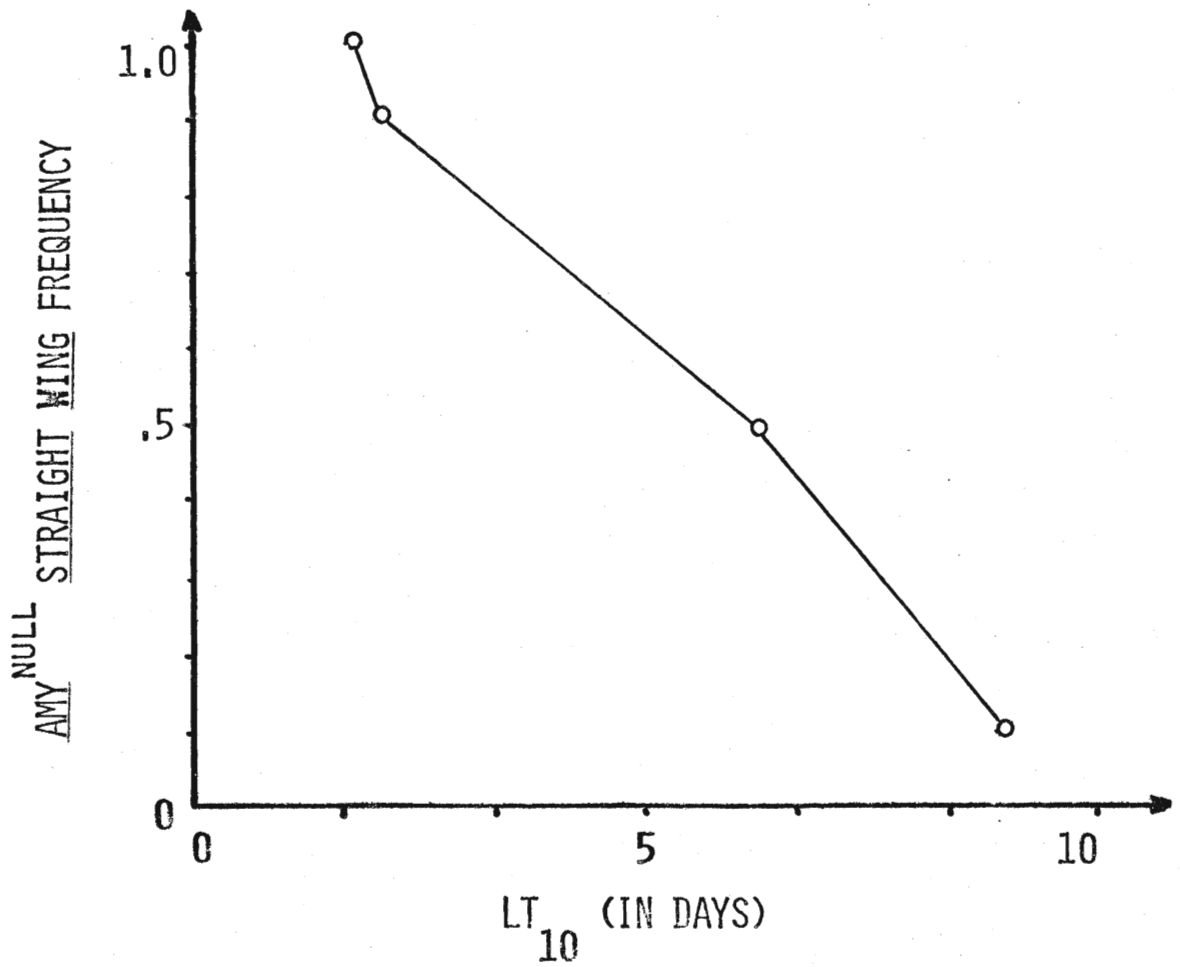

100%
50
0
AMY NULL ST.W.
AMY NULL FREQ.
.1
.5
.9
1.0
100%
50
0
AMY NULL C.W.
PERCENTAGE SURVIVORSHIP
0
2
4
6
8
10
12 TIME(DAYS

Figure 11: The LT_{10} for Amy^{null} straight wing when competing with Amy^{1-a} curly wing on starch at different frequencies. The LT_{10} is the time required till 10% of the fly population has died.

Figure 12: The LT_{10} for Amy^{null} curly wing when competing with Amy one-a straight wing at various frequencies on starch.


1.0
.5
0
0
5
10
AMY^NULL STRAIGHT WING FREQUENCY
LT10 (IN DAYS)

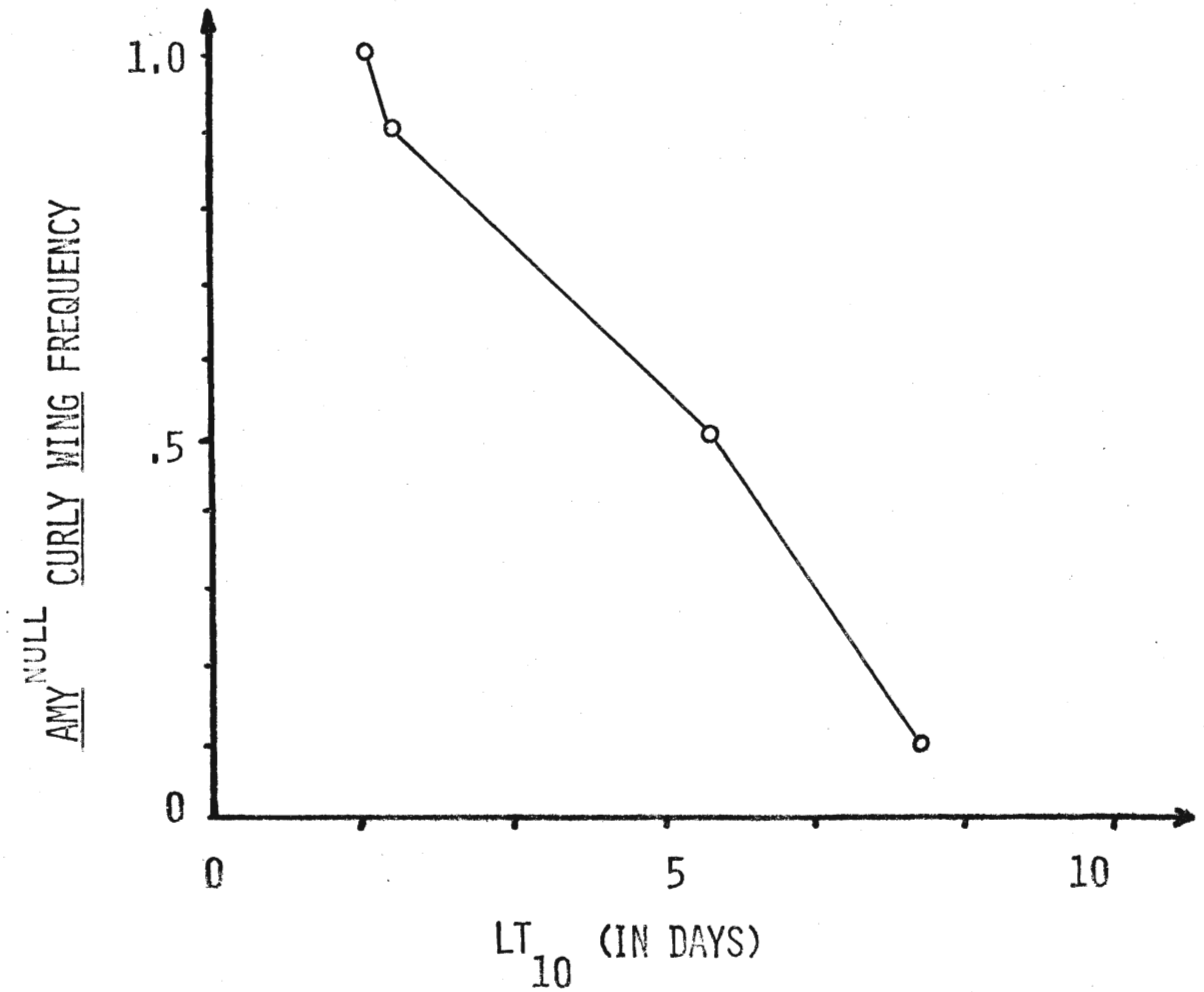

1.0
.5
0
0
5
10
AMY^NULL CURLY WING FREQUENCY
LT10 (IN DAYS)

Table 16: The mean percentage of survivorship of *Amy*^{null} *straight wing* when competing with *Amy*$^{1-a}$ *straight wing* at three different frequencies on the starch food medium (genotype was determined by gel electrophoresis).

Time in Days	Experimental Medium: 8% starch; 0.5% Brewer's yeast								Statistical Comparison (t-test)			
	Amy^{null} *straight wing* frequency											
	.1		.5		.9		1.0		.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	100	±0	100	±0	100	±0	100	±0	NS	NS	NS	NS
2	100	+0	97	±1	98	±2	96	±6	NS	NS	NS	NS
4	95	±5	97	±1	79	±7	34	±11	NS	***	***	***
6	95	±5	94	±2	57	±16	2	±2	NS	***	***	***
8	90	±10	94	±2	38	±16		-	NS	***	***	***
10	90	±10	85	±9	23	±6		-	NS	***	***	***
12	75	±15	79	±13	13	±8		-	NS	***	***	***

The transformed percentages obtained by the arcsin transformation are in the appendix, (pp. 193 , Table 26.).

NS = not significant, $P \geq 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 17: The mean percentage of survivorship of *Amy*^{null} *straight wing* when competing with *Amy*^{1}, *lobe eye* at three different frequencies on the starch food medium.

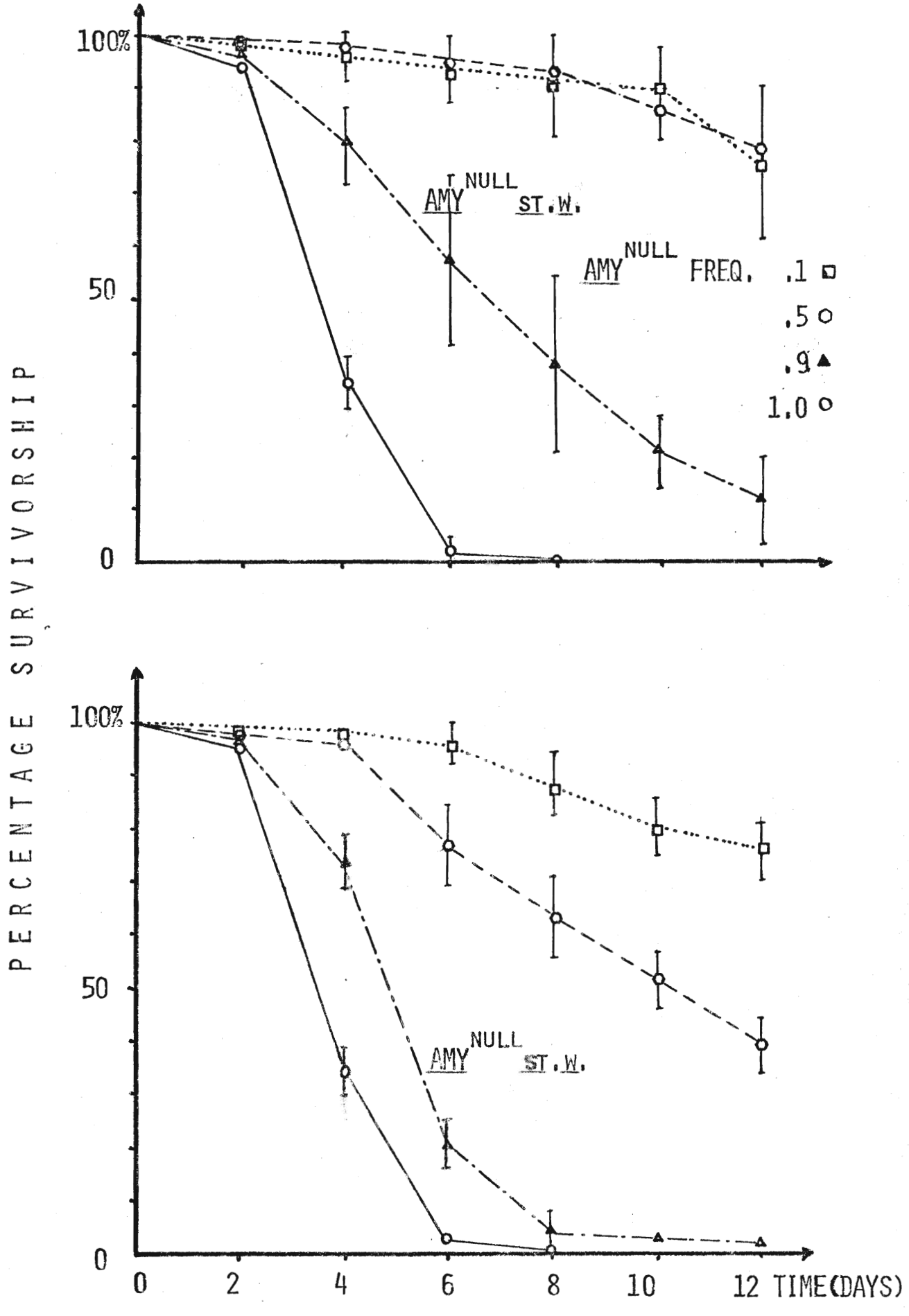
Time in Days	Experimental Medium: 8% starch; 0.5% Brewer's yeast											
	Amy^{null} *straight wing* frequency								Statistical Comparison			
	.1		.5		.9		1.0		.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	100	±0	100	±0	100	±0	100	±0	NS	NS	NS	NS
2	100	±0	99	±1	98	±1	96	±2	NS	NS	NS	NS
4	99	±1	96	±2	74	±6	34	±3	NS	***	***	***
6	96	±3	77	±7	20	±4	2	±1	**	***	***	***
8	88	±5	63	±7	5	±2		-	***	***	***	***
10	79	±5	50	±4	3	±1		-	***	***	***	***
12	66	±4	39	±5	1	±0		-	***	***	***	***

The transformed percentages obtained by the arcsin transformation are in the appendix, (pp. 194 , Table 27.).

NS = not significant, $P \geq 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Figure 13: $\underline{Amy}^{null}$ straight wing survivorship curves when the latter strain is competing with $\underline{Amy}^{1-a}$ straight wing at three different frequencies on the starch food medium. The bars represent the standard of error genotype identification was carried out by gel electrophoresis, therefore only two replicates were done. This figure represents the average of the two replicates.

Figure 14: $\underline{Amy}^{null}$ straight wing survivorship curves when the latter strain is competing with $\underline{Amy}^{1}$, lobe eye at three different frequencies on the starch food medium. These bars represent one standard of error from each side of the point.


100%
50
0
PERCENTAGE SURVIVORSHIP
AMY NULL ST.W.
AMY NULL FREQ. .1
.5
.9
1.0
AMY NULL ST.W.
0
2
4
6
8
10
12 TIME(DAYS)

Furthermore, substituting the *Amylase*$^{1-a}$ with *Amylase*^{1}, *lobe eye* did not change the outcome either. An examination of Table 17 which contains the *Amylase*^{null} mean percentage survivorship when competing with *Amylase*^{1}, *lobe eye*, shows as previously indicated that *Amylase*^{null} survivorship is frequency-dependent. For example, its survivorship at day 10 points out that when the frequency of *Amylase*^{null} is 1 its survivorship is 0%, but when its frequency is .1 its survivorship was 79 $\pm$ 5%. Figures 13 and 14 represent the *Amylase*^{null} survivorship curves when the latter strain competed with *Amylase*$^{1-a}$ and *Amylase*^{1}, *lobe eye*. Again, in these figures (13 and 14) as in the previous figures, *Amylase*^{null} survivorship is found to be frequency-dependent.

Thus, it can be concluded from this section that *Amylase*^{null} survivorship is strongly frequency-dependent regardless of the visible genetic marker being used. In other words, the lower the *Amylase*^{null} frequency in the population, the higher is its survivorship.

5) *Amylase and Sugar Excretion by Adults*

It has been shown thus far that the *Amylase*^{null} survivorship in a monoculture on a starch food medium reaches 0% within eight days. However, when *Amylase*^{null} competed with either *Amylase*$^{1-a}$ or *Amylase*^{1}, *lobe eye* on the same kind of food medium, its survivorship increased significantly; this increase in survivorship was found to be frequency-dependent. This finding is

contrary to what one would expect, since Amylase^{null} is competing for the limited amount of Brewer's yeast (0.5%) with a competitor who can utilize the available carbohydrates (starch).

The results in this section will show that the amylase-producing strains modify their environment by excreting sugar and active amylase enzymes into it. Figure 15 shows that the maltose concentration in a medium containing 8% starch and 0.5% Brewer's yeast increases as the number of amylase-producing flies (males only) increases. On the other hand, the amount of maltose detected in a medium inhabited by 100 Amylase^{null} males was found to be identical to the amount of sugar found in the control medium where no flies were raised. The raw data can be found in the appendix,(pp.195-196 , Tables 28A and B.).

Figure 16 and Table 18 point out that the Amylase^{1-a} males excreted active amylase enzymes into the medium they lived on, and that the amylase concentration increased in time. Furthermore, it is clear that on the control media, no increase in amylase activity was found (horizontal line). The raw data can be found in the appendix,(pp.197-198 , Tables 29A and 29B.).

Figure 17 and Table 19 represent the average amylase activity exhibited by one adult Amylase^{1-a} male raised on three different food media. When flies remained on the standard food medium (having 10% sucrose and 5% Brewer's yeast), their amylase activity did not change within forty-eight hours. However, when flies were transferred into a medium containing 8% starch, their

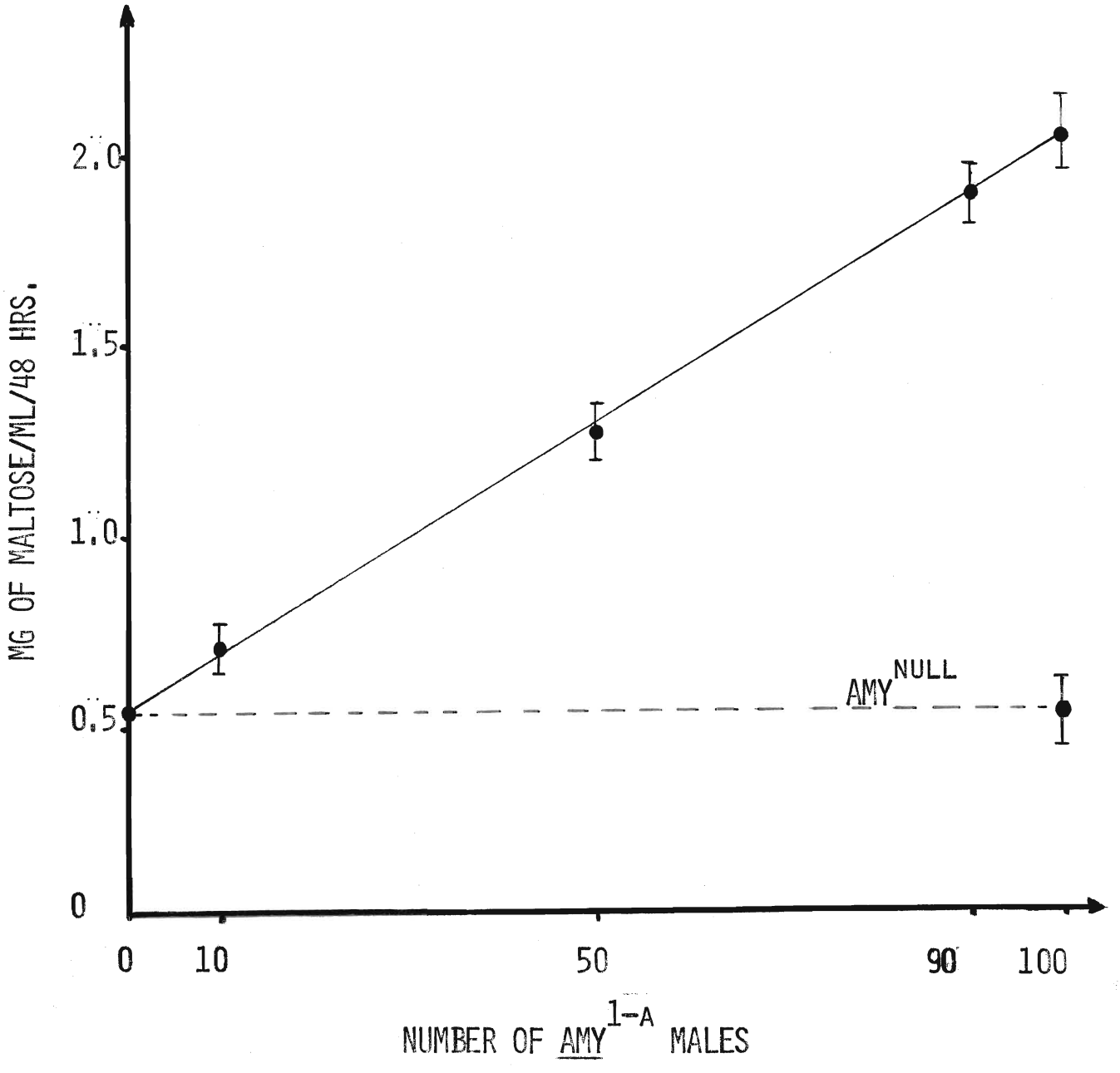


Figure 15: Maltose concentration relative to the number of Amy 1-a males after 48 hours: It can be seen that the maltose concentration increases as the number of Amy 1-a males increases. In the control (no flies) and in the medium inhabited by 100 Amy null males, the maltose concentration remained the same. The medium used in this experiment was the experimental medium (medium C).

Table 18: Absorption values reflecting amylase activity in the medium inhabited by *Amy*$^{1-a}$ males for various periods of time (on medium C.) These values represent activity per one male per thirty minutes.

Time in Hrs.	Experimental Medium: 8% starch + 0.5% B. yeast			
	Inhabited by flies		Inhabited by no flies	
	Abs. (in nm.)	Maltose*	Abs. (in nm.)	Maltose*
0	.015±.003	.051±.045	.015±.003	.051±.045
3	.018±.002	.053±.044	-	-
6	.031±.006	.061±.046	-	-
12	.034±.003	.062±.045	.010±.003	.049±.045
24	.035±.003	.063±.045	.012±.003	.050±.045
48	.082±.009	.089±.048	.012±.003	.050±.045

* Maltose Concentration (mg. of maltose/ml/1 male)

their amylase activity increased rapidly and reached a peak within six hours, then started to decrease continuously on a medium poor in Brewer's yeast (0.5%); however, it continued to increase on a medium containing 5% Brewer's yeast. The raw data can be found in the appendix, (pp.199-201 , Tables 30, 31 and 32).

Figure 18 shows that the amylase detected in the medium is

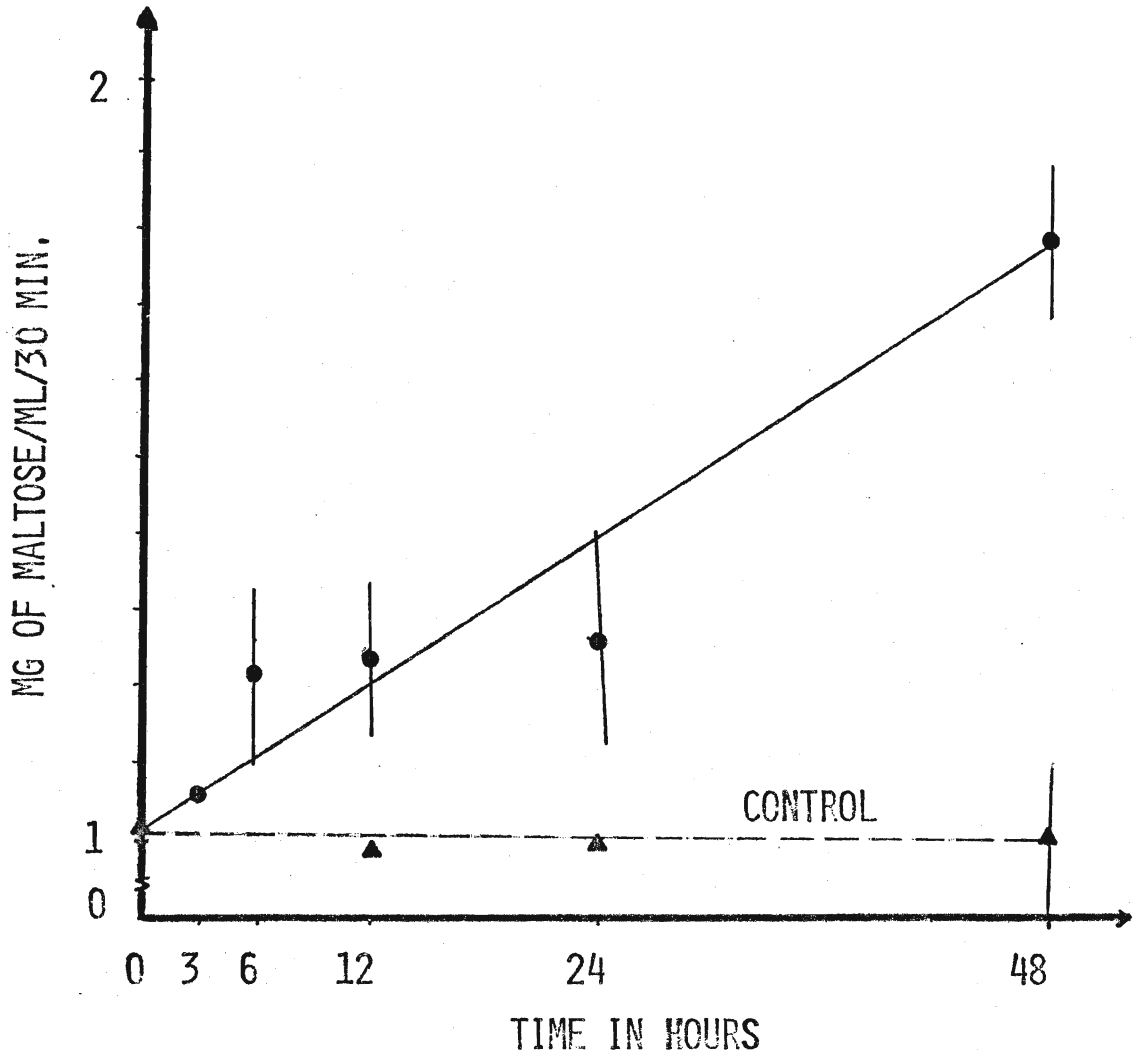


Figure 16: Average amylase activity in a medium (8% starch + 0.5% Brewer's yeast) inhabited by 20 $\frac{Amy}{1-a}$ males for various periods of time; and in a medium (8% starch + 0.5% Brewer's yeast) inhabited by no flies.

●——● Amylase activity in the experimental medium

▲- - -▲ Control: amylase activity in the experimental medium; no flies were raised on it.

Table 19: Average male amylase activity at various periods of time on two different food media. The flies' ages at time zero were between 6 and 8 days old.

Time in Hrs.	Brewer's yeast and carbohydrate concentration			
	0.5% B. yeast + 8% starch		5% B. yeast + 10% sucrose	
	Abs.(in nm.)	Maltose*	Abs.(in nm.)	Maltose*
0	.310±.010	.219±.049	.310±.010	.219±.049
3	.320±.016	.225±.052	-	-
6	.350±.062	.242±.078	-	-
12	.316±.010	.222±.049	.316±.010	.222±.049
24	.287±.010	.206±.049	.307±.010	.217±.049
48	.248±.011	.184±.049	.297±.010	.211±.049

* Maltose Concentration (mg. of maltose/ml/1 male/30 min.)

in fact Drosophila amylase, since the amylase found in the medium inhabited by male x (for example) migrated to the same distance as the amylase from whole extracts of male x itself. It is to be noted that Amylase^{null} does not have any activity on the gel, in the flies or in the excretion.

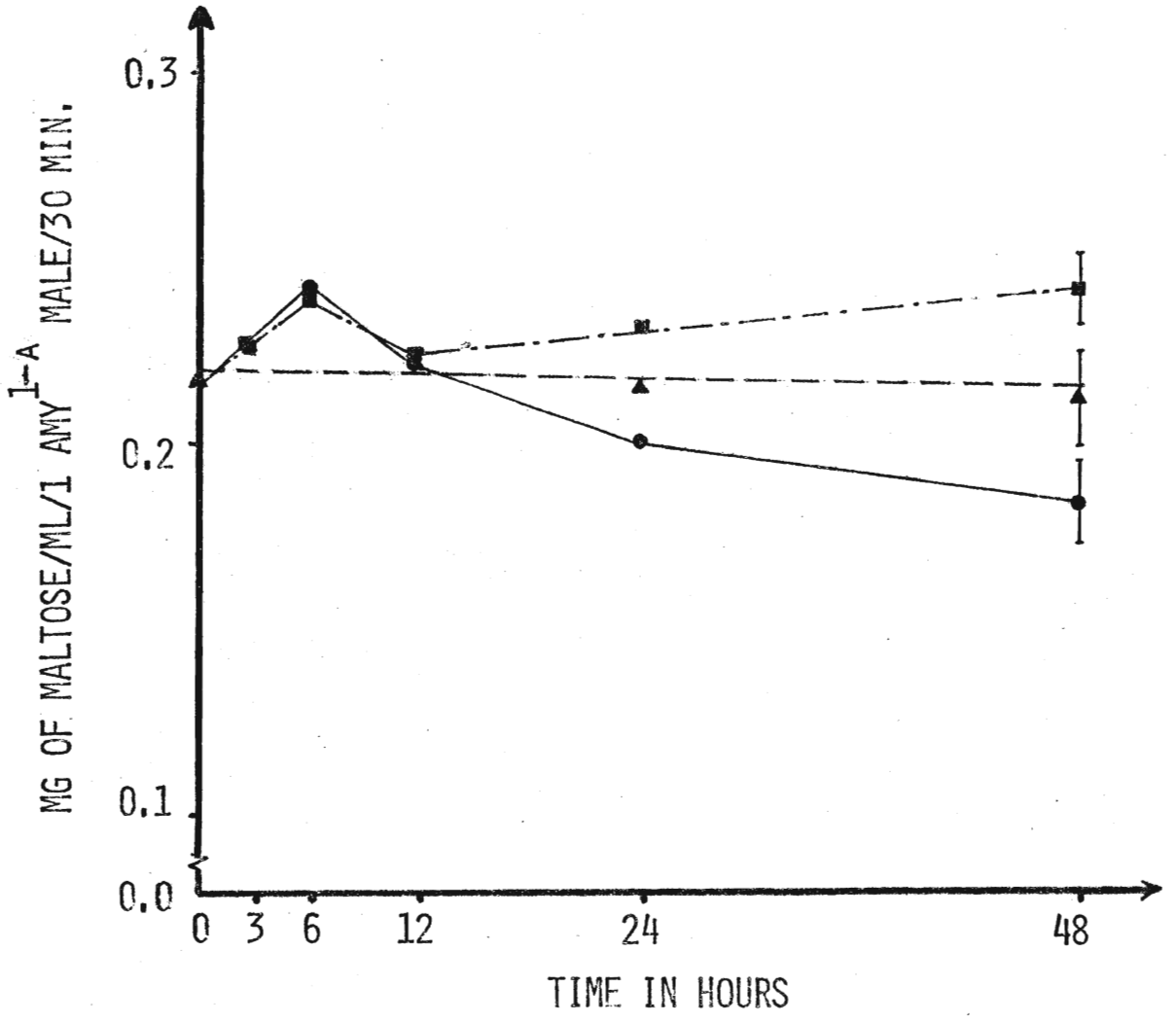


Figure 17: Average amylase enzyme activity exhibited by one adult male ($\underline{Amy}^{1-a}$) on 3 different food media:

A— - -A Male amylase activity on standard food medium (8% sucrose and 5% Brewer's yeast)

●——● Male amylase activity on food medium rich in starch (8%) but poor in Brewer's yeast (0.5%)

■— - -■ Male amylase activity on food medium rich in starch (8%) and rich in Brewer's yeast (5%)

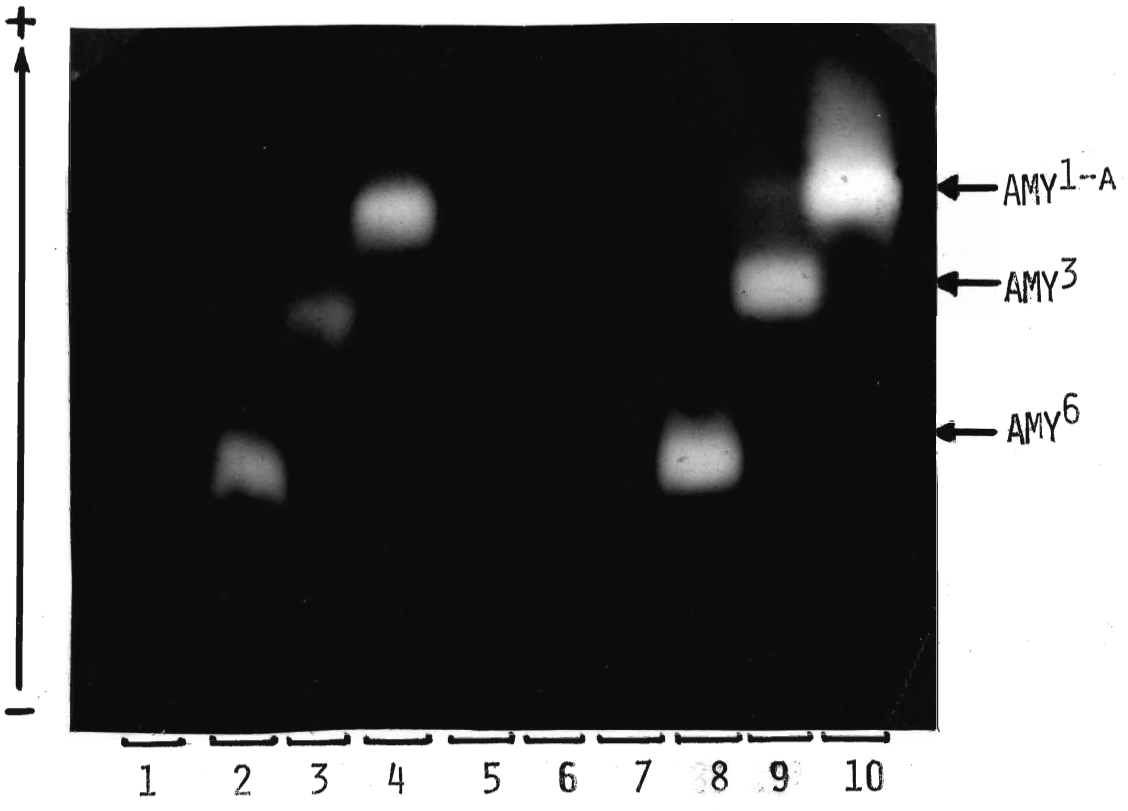


Figure 18: Electrophoretic gel picture showing that the mobility of the amylase enzymes found in the medium (packets 1 to 4) inhabited by males, is the same as the mobility of amylase from a whole male extract (7 to 10); 5 and 6 are empty packets. $\underline{Amy}^{null}$ has no activity in either the flies (packet 7) or in the excretion (packet 1). The genotype of the flies used is indicated to the right of the figure; the arrow to the left indicates the direction of the enzyme migration.

6) Amylase and Sugar Excretion by Larvae, and their Carbohydrate Requirements

Larvae modify the environment they inhabit just as the adults do. The first part of this section deals with the larval maltose and amylase excretion; the second part deals with larval viability and carbohydrate requirements.

a) Larval Sugar and Amylase Excretion:

Figure 19 which represents the maltose concentration in the medium inhabited by third instar Amylase^{1-a} larvae, and in the control food medium, shows that the absorption increases (which corresponds to the maltose concentration) in the medium inhabited by the Amylase^{1-a} third instar larvae. In the medium with no larvae (the control medium) no such increase can be seen.

The larvae also excreted active amylase enzymes into the medium they live on. Figure 20 shows that in the control medium as well as in the medium inhabited by the Amylase^{null} third instar larvae, no increase in amylase activity was detected (the two horizontal lines at the bottom of the figure). However, amylase activity increases in the medium inhabited by Amylase^{1-a} larvae, gradually in time and seems to level off after sixteen hours. This increase in amylase activity (in the third instar larvae) reaches a peak within four hours and then starts to decrease gradually thereafter. The raw data for this section can be found in the appendix, (pp.202-204 , Tables 33 to 35).

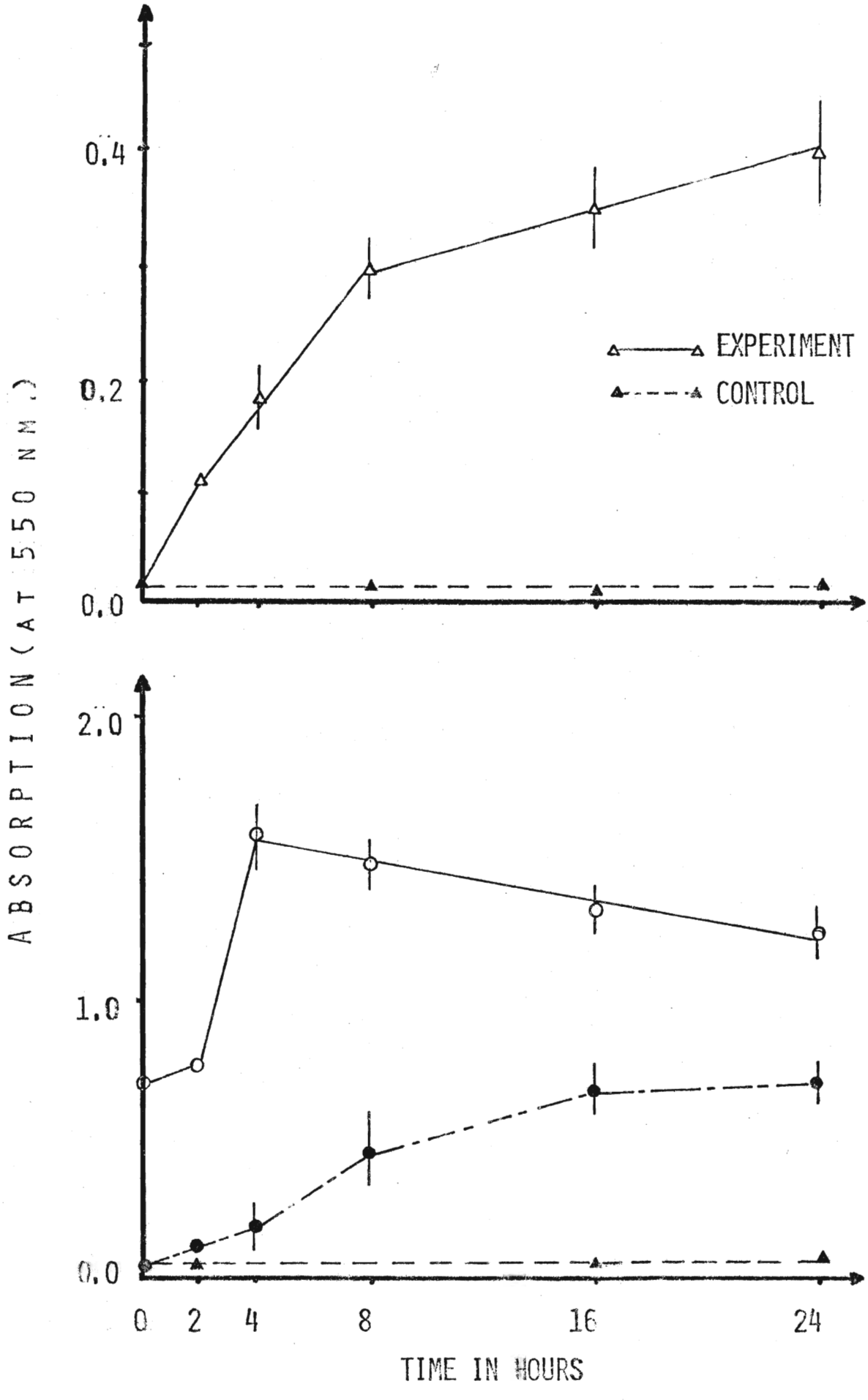
Figure 19: Maltose concentration in the medium inhabited by the third instar Amylase$^{1\text{-}a}$ larvae, and in the medium inhabited by no larvae as a control.

Figure 20: Amylase activity in the third instar Amy$^{1\text{-}a}$ and Amy^{null} larvae, and in the medium they inhabited for various periods of time. The two bottom lines represent the control and the Amy^{null} amylase activity.

o———o Amylase in larvae

●———● Amylase in larval excretion

▲- - -▲ Amylase in control medium


0.4
0.2
0.0
EXPERIMENT
CONTROL
2.0
1.0
0.0
ABSORPTION (AT 550 NM.)
0
2
4
8
16
24
TIME IN HOURS

Gel electrophoresis (Figures 21 and 22) confirms that the amylase found in the medium inhabited by a given strain of larvae, originates from these larvae. $\underline{Amylase}^{null}$ larvae again do not show any amylase activity. The gel scan (Figure 23) which shows amylase activity in the mixtures of homozygous ($\underline{Amylase}^{6}$ + $\underline{Amylase}^{1-a}$) and F_1 heterozygous ($\underline{Amylase}^{6}$ / $\underline{Amylase}^{1-a}$) third instar larvae and in their excretion, reveals that more amylase from $\underline{Amylase}^{1-a}$ larvae was excreted than from $\underline{Amylase}^{6}$ (Figure 23). This difference in excretion is more evident in the mixture of homozygotes. For example, let us examine the peak height ratio of $\underline{Amylase}^{1-a}$ to $\underline{Amylase}^{6}$ for the larvae and for the excretion. In F_1 heterozygous ($\underline{Amylase}^{1-a}$ / $\underline{Amylase}^{6}$) third instar larvae, the $\underline{Amylase}^{1-a}$ to $\underline{Amylase}^{6}$ peak height ratio is 1, whereas the ratio of $\underline{Amylase}^{1-a}$ to $\underline{Amylase}^{6}$ in the excretion is 4. In the mixture of homozygotes ($\underline{Amylase}^{1-a}$ + $\underline{Amylase}^{6}$), the $\underline{Amylase}^{1-a}$ to $\underline{Amylase}^{6}$ peak height ratio is 1 which is very similar to the one observed for the heterozygotes. However, in the excretion, the $\underline{Amylase}^{1-a}$ to $\underline{Amylase}^{6}$ peak height ratio is 30. In other words, $\underline{Amylase}^{1-a}$ is present in the excretion of the heterozygotes ($\underline{Amylase}^{1-a}$ / $\underline{Amylase}^{6}$) 4 times more than $\underline{Amylase}^{6}$, whereas in the mixture of homozygotes, $\underline{Amylase}^{1-a}$ is 30 times more abundant than $\underline{Amylase}^{6}$.

Figure 24 shows the amylase distribution along three genotypic guts ($\underline{Amylase}^{6}$, $\underline{Amylase}^{1-a}$ and $\underline{Amylase}^{1-c}$). It can be seen that the amylase activity (white area) in $\underline{Amylase}^{6}$ adults is distributed from around the head down to the posterior part of the

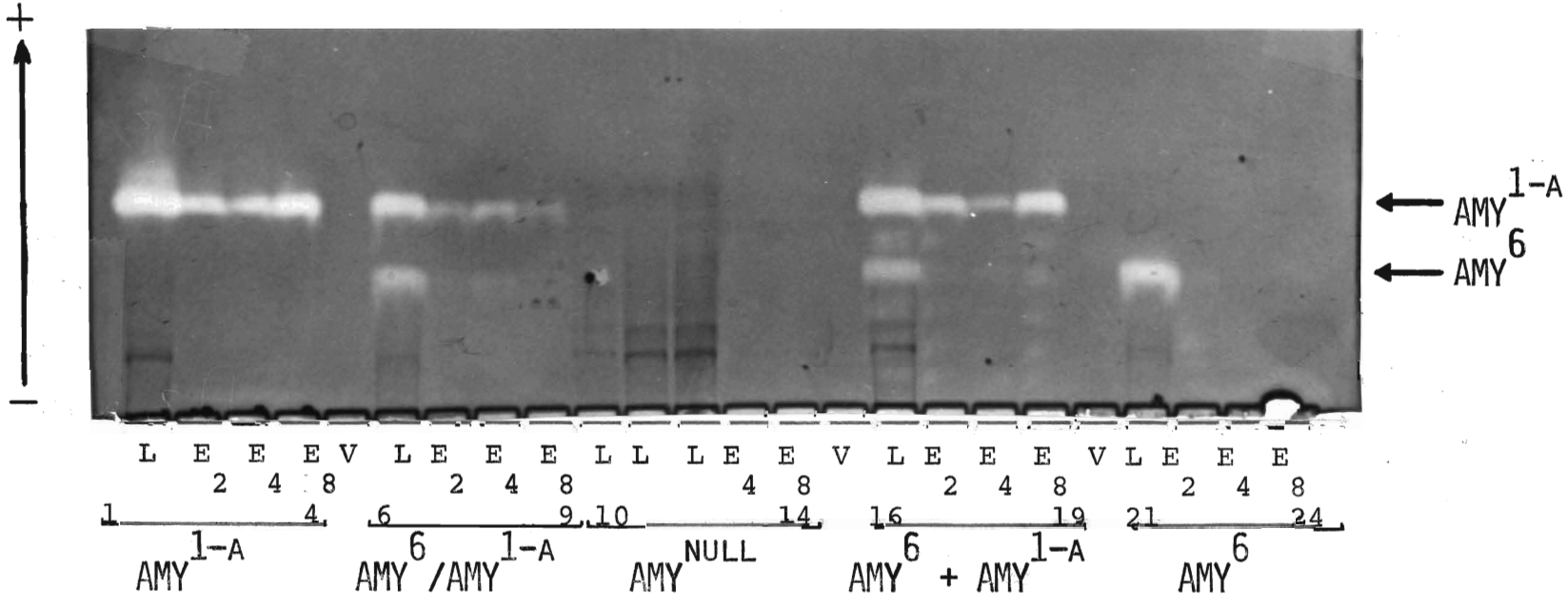


Figure 21: Amylase in third instar larvae and in their excretion: Genotypes are indicated below and to the left of the picture; L is the banding pattern produced by extracts from the larvae, E is the banding pattern produced by their excretion and V is an empty packet. The arrow to the left of the picture indicates the direction of migration. $\underline{Amy}^{null}$ (11 to 14) did not show any amylase activity. Furthermore, the amylase found in the medium increases in time: on E_2 the larvae were left for two hours, on E_4 for four hours and on E_8 for eight hours.

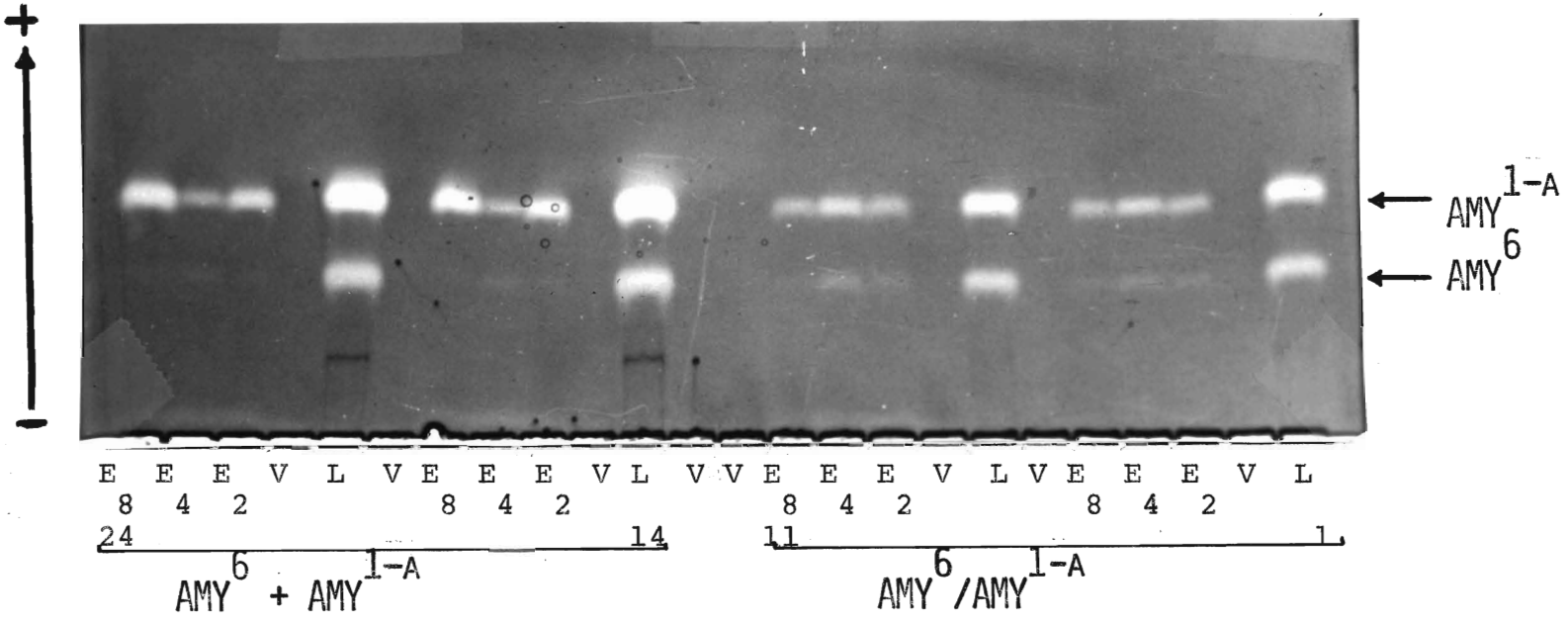


Figure 22: Amylase in third instar (Homozygotes 14 to 24, and Heterozygotes 1 to 11) larvae and in their excretion: L is the banding pattern produced by extracts from the third instar larvae, E is the banding pattern produced by their excretion and V is an empty packet. Genotypes are indicated below and to the right of the picture. The arrow to the left of the figure indicates the direction of migration.

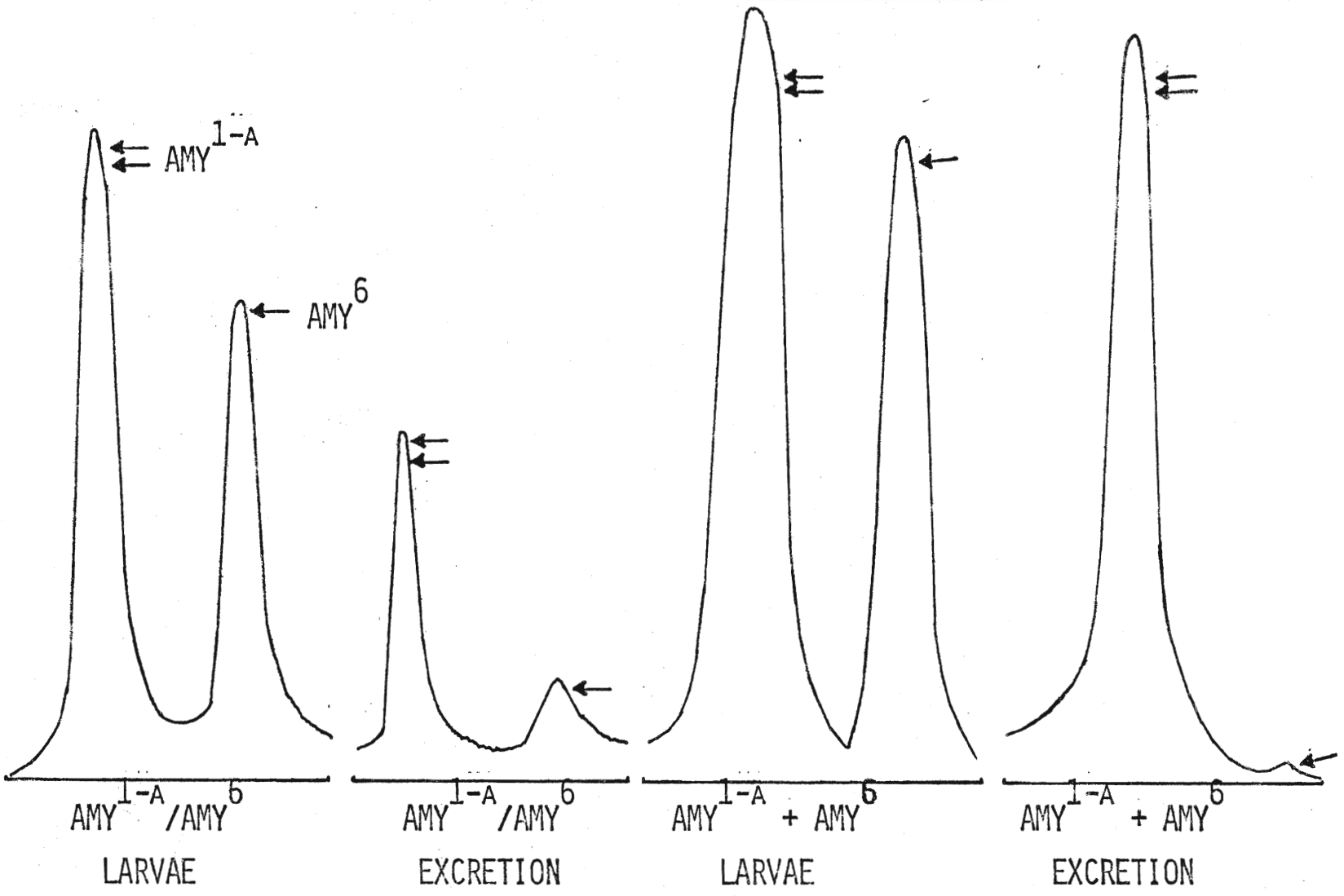


Figure 23: Comparison of amylase activity in mixture of homozygotes and heterozygotes, and in their excretion: A densitometer chart was obtained by scanning the gel in Figure 22.

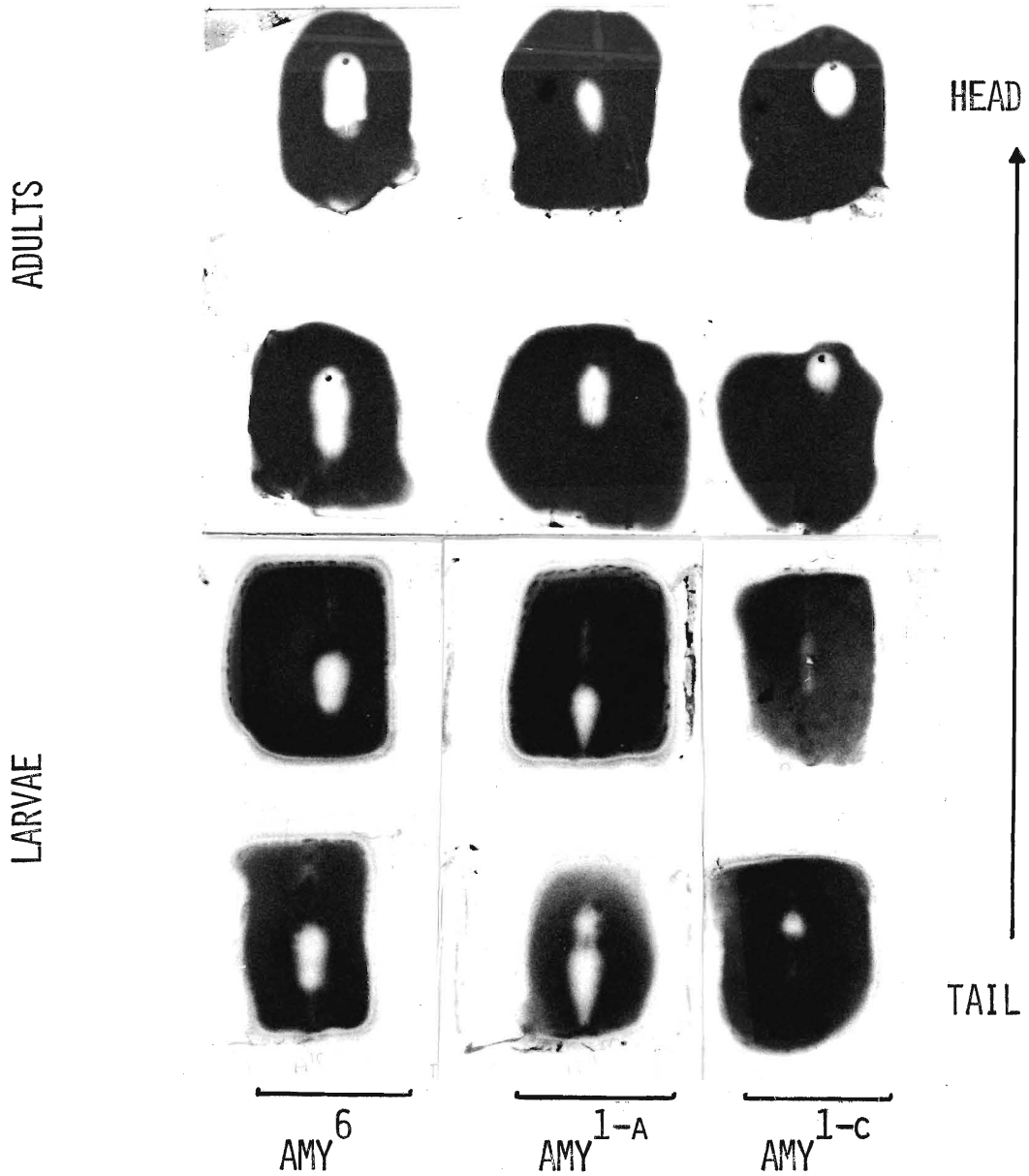


Figure 24: Amylase distribution along the gut of adults and larvae: The amylase activity is the white area along the gut, with the head of the fly or larva at the top, and the rear end at the bottom.

midgut; whereas in Amylase^{1-a} the activity is seen to be centered in the anterior and midgut areas. Adult Amylase^{1-c} activity seems to be located in the anterior portion of the midgut.

In larvae, the picture is different. Amylase^{6} shows some activity in the posterior portion of the midgut, whereas Amylase^{1-a} in one gut shows activity in the posterior portion of the midgut while in the second gut, amylase activity seems to be distributed in the anterior and posterior portions of the gut with little activity in the middle portion of the midgut. The distribution of amylase activity in Amylase^{1-c} larvae seems to be along the posterior portion of the midgut.

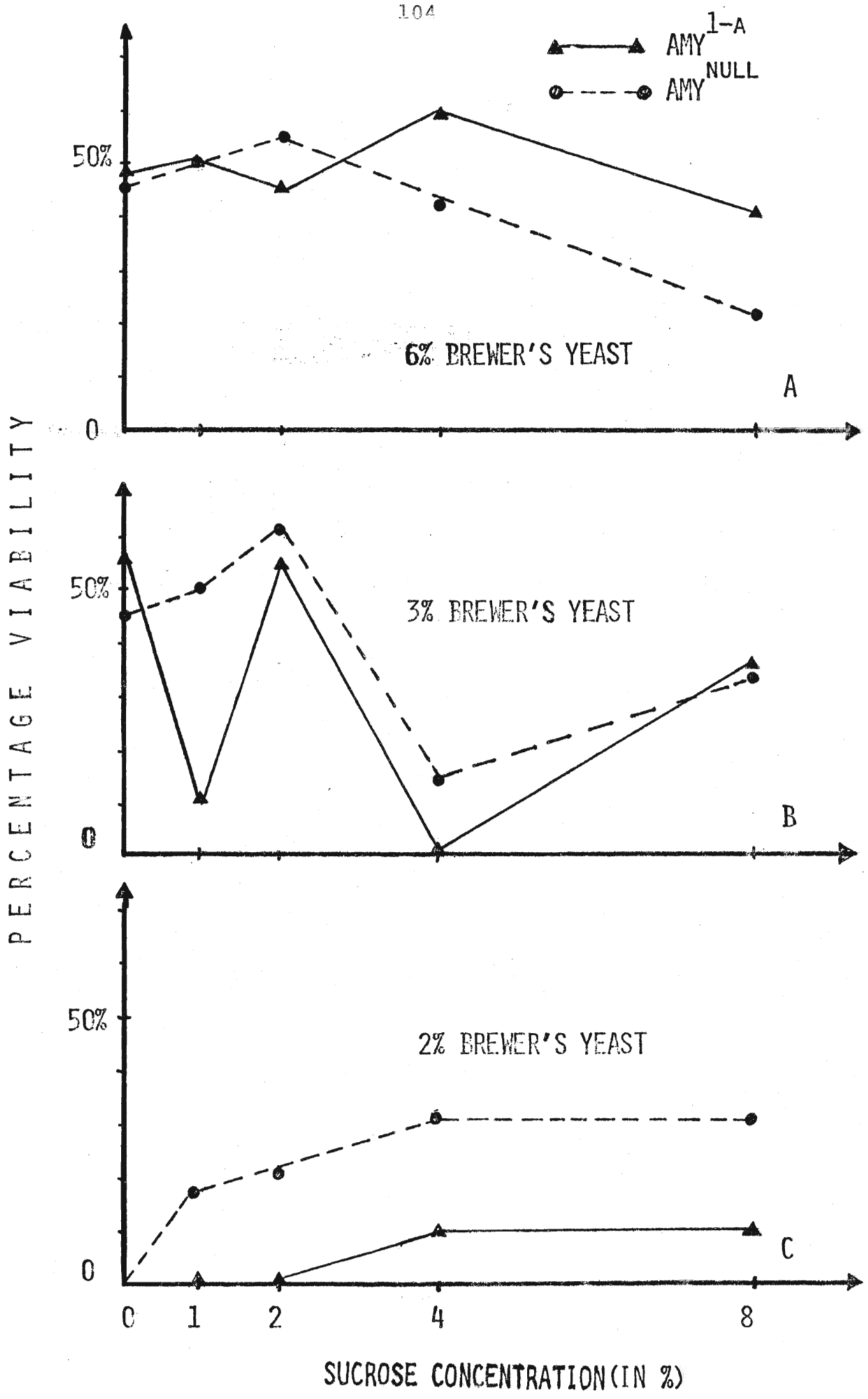
b) Larval Carbohydrate Requirements and Viability:

Figures 25A, 25B and 25C represent the mean percentage egg-to-adult viability on fifteen different food media. Figure 25A represents the percentage of eggs that become adults on five media, all having 6% Brewer's yeast but differing in sucrose concentrations (0%, 1%, 2%, 4% and 8%). It is apparent that at this level of Brewer's yeast concentration (6%), the increase in sucrose concentration seems to have a negative effect on the egg-to-adult viability. However, when the amount of Brewer's yeast was reduced to 3%, the results were mixed (Figure 25B); nevertheless, a 2% sucrose concentration seems to give the highest egg-to-adult viability (60%). When the concentration of Brewer's yeast was reduced to 2% (Figure 25C), a minimum of a 1% sucrose concentration was required before any egg could reach adulthood. It is to be

Figure 25A: Egg-to-adult viability of $\underline{Amy}^{1\text{-}a}$ and $\underline{Amy}^{null}$ on five media, all having 6% dead Brewer's yeast but differing in sucrose concentrations (0%, 1%, 2%, 4% and 8%). Raw data are in the appendix,(Table 36A, p. 205).

Figure 25B: Same as the above except that the concentration of dead Brewer's yeast was reduced to 3%. Raw data are in the appendix,(Table 36B, p. 206).

Figure 25C: Same as in 31A except that the concentation of Brewer's yeast is 2%. The raw data are in the appendix, Table 36C. It is apparent that at a low concentration (2%) of Brewer's yeast, a minimal amount of carbohydrates is required for the eggs to become adults.


AMY 1-A
AMY NULL
50%
0
6% BREWER'S YEAST
A
50%
3% BREWER'S YEAST
0
B
50%
2% BREWER'S YEAST
0
C
0
1
2
4
8
PERCENTAGE VIABILITY
SUCROSE CONCENTRATION (IN %)

noted that at this concentration of Brewer's yeast, at the most, 30% of the eggs reached the adult stage, whereas at the 3% and 6% concentrations of Brewer's yeast, the maximum viability was 60%.

Finally, from this section one could conclude that larvae excrete sugar and amylase just as adults do (Figures 19, 20, 21 and 22), and that more Amylase $^{1\text{-}a}$ enzymes are excreted than Amylase ^{6} enzymes (Figure 23). Furthermore, the amylase distribution along the adult and larval guts varies (Figure 24). In addition, a high sugar concentration is seen to have a negative effect on egg-to-adult viability at a high yeast concentration; however, the opposite is true at a low yeast concentration.

VI DISCUSSION

The lack of a well documented study on how frequency-dependent selection functions (Clarke, 1979), and more specifically at one enzyme locus, as well as the availability of a newly discovered amylase deficient variant of Drosophila melanogaster (Hickey, 1980) induced me to investigate frequency-dependent selection at the amylase locus. Special attention was given to the biochemistry of the media on which the fitness (survivorship) of an amylase-producing strain and an amylase deficient strain was measured.

The great difference in amylase enzyme activity between the amylase-producing and amylase deficient strains led one to predict that at a low yeast level, the addition of starch would promote the survivorship of the amylase-producing strains while the Amylase null genotype would not benefit from it and its survivorship would remain low. Thus, the amylase-producing strains would have a definite advantage in terms of survivorship, over the amylase deficient strain. This prediction would remain true whether the amylase deficient and amylase-producing strains were raised in a pure culture or competed in a mixed culture. One would expect this survivorship advantage to increase as the concentration of starch increases and the level of Brewer's yeast decreases. On the other hand, this advantage would decrease as the concentration of starch

decreases and the level of Brewer's yeast or simple sugars increases.

Before proceeding, I would like to point out that the current study is the only one which measures fitness at one enzyme locus in a short period of time (twelve days) in a single generation, at the adult stage. Similar studies to date have measured selection either in a dozen generations (Tobari and Kojima, 1967; Anxolabéhère, 1976; Hickey, 1977, 1979, and others) or from the egg to the adult (Dawood and Strickberger, 1969; Huang et al, 1971; DeJong and Scharloo, 1976; Snyder and Ayala, 1979; Nassar, 1980; Caligari, 1980, and others). In addition, restricting the study to a homozygous adult genotype eliminated the problem of heterosis (Snyder and Ayala, 1979) and rare male mating advantage.

Adult Survivorship on Nine Different Food Media:

The results obtained by testing the survivorship of null 4,6
Amylase and Amylase on nine different food media (Table 1) strongly supports the above prediction. Figures 1A, 1B and 1C 4,6
show that while Amylase survivorship was increasing (as the concentration of Brewer's decreased) from 90% survivorship null
(Figure 1A) to almost 100% survivorship (Figure 1C), Amylase survivorship decreased from 85% (Figure 1A) to 0% (Figure 1C). null
The decrease in Amylase survivorship was expected since it cannot utilize the available carbohydrate (starch); this is due primarily to the lack of amylase activity. However, the increase 4,6
in Amylase survivorship as the concentration of Brewer's yeast

decreased (from 8% to 0.5%) can be attributed to either microbial contamination and/or to the fact that a high concentration of yeast (8%) has a negative effect on survivorship when combined with a high starch concentration. De Jong and Scharloo (1976) reported that the combination of high yeast and high starch levels has a negative effect on larval viability; however, they did not offer any explanation why this is so. One could attribute this negative effect to the by-products because at a high yeast level, the flies would consume more proteins and thus would excrete more waste material which could be more harmful to some genotypes than others. Accordingly, at a low yeast concentration, protein consumption would be low and therefore the excretion of the by-products would also be low, thus the medium would be more favourable.

Budnik and Prncic (1976) reported that the metabolic by-products of some genotypes are in some cases harmful to the same genotype which excreted them, and in some other cases are not. Furthermore, when Huang et al (1971) and Kojima and Huang (1972) attempted to elucidate the mechanism of frequency-dependent selection, they found that the relative viability of a given genotype was the lowest when raised on a medium conditioned by larvae of the same genotype, and highest when conditioned by larvae of a different genotype. They concluded that this is due to either the depletion of the required nutrients or to the excretion of the metabolic by-products. My results show that the Amylase[4,6] survivorship in a monoculture increased as the concentration of Brewer's yeast decreased. In other words, this finding allows one

to reject the first part of Huang et al (1971) and Kojima and Huang (1972) 's conclusion, and to support the second part, i.e., the by-products are responsible for the observed interference or facilitation and not the depletion of nutrients.

Reducing the starch concentration from 8% (Figures 1A, 1B, 1C) to 2% (Figures 2D, 2E and 2F) did not change appreciably the overall outcome of the experiment. This is again due to the same reasons stated previously.

On the last three food media (G, H and I from Table 1), one would expect to see that no difference exists between the survivorship of the amylase-producing and amylase deficient strains. This is because on these three media (G, H and I) there is no starch present, thus the possession of amylase activity on these media becomes irrelevant. In other words, the difference in survivorship which exists on these food media would be attributed to other factors than the amylase locus. Figures 3G, 3H and 3I point out that the survivorship of the three genotypes (Amylase^{null}, Amylase^{1} lobe eye and Amylase^{1-a}) of Drosophila melanogaster is a function of the Brewer's yeast concentration, i.e., when the yeast level decreases, survivorship also decreases. This is true regardless of whether these genotypes have amylase activity or not.

Let me now address the question of why the amylase-producing and amylase deficient strain survivorship curves were not identical on these three food media (G, H and I). This difference in survivorship was found to be significant in some cases; for

example, at days 8 and 10 (Figures 3G and 3H), Amylase^{4,6} survived significantly better ($P<.05$, $t = 2.39$) than Amylase^{null}. This can be attributed to a number of reasons some of which have been stated previously (e.g. by-products). In the first place, Amylase^{4,6} not only differs from Amylase^{null} at the amylase locus, but at various other loci. Secondly, the number of flies used in these replicates is low (ten flies per vial); thus, if one single fly dies, this means having a mortality of 10%. Furthermore, only five replicates were done. Figure 3I supports this argument; where ten replicates were carried out and 100 flies per vial were used, the difference between the amylase-producing and amylase deficient strains in survivorship decreased and became insignificant. Lastly, the survivorship experiment on the nine food media served its purpose by allowing us to pinpoint the medium which amplifies selective pressure against Amylase^{null} without affecting the amylase-producing strains.

Survivorship at Various Densities:

It has been pointed out by various authors that density affects Drosophila survivorship (or fitness) (Chiang and Hodson, 1950; Lewontin, 1955; Alvarez et al, 1979, and others). However, My results did not show density-dependent survivorship. This is primarily due to the fact that I did not use extreme densities such as 1000 flies per vial or very few flies per vial. I used 100 flies per vial as the high density and 10 flies per vial as the low density. Thus, it seems that 100 flies per vial is not a high density, nor is 10 flies per vial a low density in the vials which

were employed (2 x 10 cm.).

The survivorship curves for Amylase^{null}, Amylase$^{1\text{-}a}$ and Amylase^{4,6} at four densities for each genotype on starch (8% and 0.5% Brewer's yeast) food media (Figures 4A, 4B and 4C respectively) point out that survivorship was independent of density at these particular densities. This seems to be contrary to what one would expect; for example, even though 100 flies per vial and 10 flies per vial are not extreme densities, the former is still ten times greater than the latter density, and therefore one would predict that at the low density (10 flies per vial), the flies would survive relatively longer than at the high density (100 flies per vial). This should be true in particular for Amylase^{null} since it cannot utilize the available starch (8%), but individual flies will compete for the limited amount of available yeast (0.5%). This did not happen, as all of the Amylase^{null} flies died within eight days (Table 8) regardless of the density used. However, density did seem to have an effect on Amylase^{null} survivorship at days 2 and 4 (Table 8, experimental medium). For example, on day 2 at the low density (10 flies per vial), 100% survivorship was recorded whereas at the high density (100 flies per vial) 80% survivorship was observed. Furthermore, on day 4, 50% survivorship was recorded at the low density versus 30% survivorship at the high density for the same genotype on the same medium (experimental medium).

Since the purpose of this experiment was not an in-depth investigation of density-dependent selection, but rather to allow

me to select an appropriate density for further experiments on frequency-dependent selection at the amylase locus, 100 flies per vial was chosen for reasons previously stated; nevertheless, the obvious reason was that at the lower densities, the mortality of a few flies (accidently for example) represents a large percentage. For instance, in Table 10, Amylase^{4,6} survivorship at the low density (10 flies per vial) on glucose after ten days was 90% whereas on starch it was 70%; this difference could be due to the accidental death of simply one fly in one case, and three flies in the other. On the other hand, looking at survivorship at the high density (100 flies per vial) for the same period of time shows that Amylase^{4,6} survivorship on glucose was 88% and 87% on starch.

Amylase-Producing and Amylase Deficient Variant Survivorship in a Monoculture on Starch and Glucose Food Media:

After having selected an appropriate experimental medium (medium C) and an appropriate density, finding a suitable control medium was not a problem, since the amylase substrate (primarily starch) is well known and the final by-product of the enzyme (glucose) is also known. Therefore, the control food medium consisted of exactly the same composition as the experimental medium (8% starch and 0.5% Brewer's yeast) except that the substrate, starch, was replaced by the final by-product, 8% glucose. On the control food medium, one would expect the survivorship of the amylase-producing and amylase deficient strains to be the same; this is because amylase activity on this medium is irrelevant to survivorship. Amylase occurs in a number of tissues and in the

hemolymph, but it is present primarily in the midgut of Drosophila melanogaster. The function of amylase in the midgut is the digestion of nutritional starch, but one has to point out that the function of amylase in the hemolymph and other tissues is unknown (Doane, 1969b).

The outcome of the monoculture experiments on the control food medium agrees with the notion of amylase locus irrelevance to the degree of survivorship. Figure 8A testifies to the veracity of the above statement. In other words, the survivorship of the three genotypes on the control food medium was remarkably similar and over 95%.

On the experimental food medium the picture is different. Survivorship was very closely related to the amylase locus. Figure 8C shows that Amylase^{1-a} did not survive significantly better than Amylase^{1} lobe eye whereas Amylase^{null} survivorship rapidly declined to extinction within eight days. This rapid increase in the mortality rate on the experimental medium suggests that the competition between the Amylase^{null} individuals can be classified as scramble competition as described by Nicholson (1954). In scramble competition, the available resources are shared equally among all the competing individuals; when the resources per individual fly became insufficient for survival, the mortality rate rose from 0% to 100% within a few days.

Amylase^{null} females survived generally better than the males, however, this difference was significant only on day 4 of

the experiment (Figures 7A and 7B). This is due to the difference in body weight; females have a larger body size than do males. On the experimental medium, *Amylase*^{null} flies cannot utilize starch as a source of energy because they lack amylase activity. Further, the level of yeast (0.5%) is too low to keep them alive for more than a few days; consequently, they are forced into a fasting state. In a fasting state, the flies will rapidly lose most of their adipose tissues, then most of their protein in order to provide glucose for their vital organs such as the brain. Thus, the larger the fly, the longer it lives under fasting conditions.

Now let us compare the survivorship of the amylase-producing strains on the experimental and control food media (Figures 5A and 6). Although *Amylase*$^{1-a}$ and *Amylase*^{1} *lobe eye* can utilize starch, their survivorship on the experimental medium which contained 8% starch, was found to be lower than on the control food medium which contained 8% glucose. This can partially be attributed to the fact that the experimental medium is poor in yeast (source of amino acids), for the utilization of starch requires the biosynthesis of amylase enzymes whose production is dependent upon the availability of amino acids; this is clearly seen in Figure 17. It has been shown that amylase activity in *Drosophila melanogaster* is significantly higher on a starch rich diet than on a glucose or sucrose, or sucrose plus starch rich diet (Doane, 1969a). Hoorn and Scharloo (1978) pointed out that the ultimate starch effect depends on other components such as the yeast level in the medium. The results in Figure 17 support

Hoorn and Scharloo (1978) 's conclusion; it is evident that the flies on a starch and yeast rich medium have higher amylase activity than the flies raised on a sucrose and yeast rich diet. However, on a medium poor in yeast (0.5%), the level of amylase activity decreased steadily in time in spite of the presence of 8% starch.

In addressing the question of whether genetic variation is maintained by balanced selection or is the result of random genetic drift, the neutralist assumes variation to be selectively neutral (Kimura and Ohta, 1971). In comparing the survivorship of the genotype with the higher amylase activity ($\underline{\text{Amylase}}^{1-a}$) to the genotype with the lower amylase activity ($\underline{\text{Amylase}}^{1}$ $\underline{\text{lobe}}$ $\underline{\text{eye}}$) (Figure 8C), it can be seen that $\underline{\text{Amylase}}^{1-a}$ survived better than $\underline{\text{Amylase}}^{1}$ $\underline{\text{lobe}}$ $\underline{\text{eye}}$. This led one to conclude that the amylase enzyme variants are not functionally neutral, and that $\underline{\text{Amylase}}^{1-a}$ has a higher fitness than $\underline{\text{Amylase}}^{1}$ $\underline{\text{lobe}}$ $\underline{\text{eye}}$ on this food medium. This has been confirmed by Hickey (1977) and Scharloo et al (1977) who reported that amylase enzyme variants are not functionally equivalent.

Thus far, I have shown that in a monoculture at a low yeast level (0.5%), in the presence of 8% glucose (control medium), no difference in survivorship exists between the strains tested, while on the experimental medium (0.5% yeast and 8% starch) a difference in survivorship does exist. The $\underline{\text{Amylase}}^{1-a}$ strain survived better than the $\underline{\text{Amylase}}^{1}$ $\underline{\text{lobe}}$ $\underline{\text{eye}}$ variant, and both did significantly better than $\underline{\text{Amylase}}^{\text{null}}$. This is the consequence

of the great difference in enzymatic activity with *Amylase*$^{1-a}$ having higher enzymatic activity than *Amylase*^{1} *lobe eye*, and both having infinitely more enzymatic activity than the *Amylase*^{null} variant. This permits one to conclude that selection was acting directly on the amylase locus. Selection at one enzyme locus has been demonstrated by De Jong and Scharloo (1976), Hickey (1977), Snyder and Ayala (1979) and Nassar (1980). Nevertheless some of these studies have been subject to criticism (Yardley, 1978), since it is not possible to know whether selection was acting on these enzyme loci or on the general genetic background. The results reported in this thesis overcome this criticism and indicate clearly that selection was acting on the amylase locus.

Intraspecific Competition on Starch and Glucose Food Media:

The importance of frequency-dependent selection in maintaining genetic polymorphisms has been recognized and discussed by a number of authors (Ayala and Campbell, 1974; Spiess, 1977; Clarke, 1979, and others). Nevertheless, in experimental population genetics only a few studies sought to detect it at allozyme loci. Kojima and his co-workers (Huang et al, 1971; Kojima and Yarbrough, 1967; Kojima and Tobari, 1969b) have been successful in presenting evidence for frequency-dependent selection at the esterase-6 and Adh loci of *Drosophila melanogaster*. Furthermore, Snyder and Ayala (1979), Nassar (1980) and others presented evidence of frequency-dependent selection at the PGM-1 and LAP loci respectively. Nonetheless, the mechanism responsible for this selective mode remains unknown (Clarke, 1979).

Selection has been shown to act on the amylase locus in a monoculture on the experimental medium, favouring greatly the amylase-producing strains (*Amylase*$^{1\text{-}a}$ and *Amylase*^{1} *lobe eye*) over the amylase deficient strain (*Amylase*^{null}). The question then arises of whether *Amylase*^{null} survivorship (fitness) will increase or decrease when competing in a mixed culture on the experimental medium with either *Amylase*$^{1\text{-}a}$ or *Amylase*^{1} *lobe eye*. Naturally, one would expect *Amylase*^{null} survivorship to remain the same as in the monoculture or to decrease which is more likely to happen, since it is competing for a limited amount of Brewer's yeast with either *Amylase*$^{1\text{-}a}$ or *Amylase*^{1} *lobe eye*. Furthermore, one would predict that this expectation would hold true regardless of the *Amylase*^{null} frequency used.

The outcome of the competition experiments disagree totally with my prediction. In other words, *Amylase*^{null} survivorship was found to be frequency-dependent on the experimental medium. For example, when *Amylase*^{null} *straight wing* competed with *Amylase*$^{1\text{-}a}$ *curly wing*, *Amylase*^{null} survivorship at day 10 increased from 12 $\pm$ 3% when its frequency was .9 to 86 $\pm$ 3% when its frequency became .5, and to 95 $\pm$ 3% when its frequency decreased to .1 (Table 14A). Reversing the visible genetic marker did not change the outcome of the experiment. Table 15 points out that when *Amylase*^{null} *curly wing* competed with *Amylase*$^{1\text{-}a}$ *straight wing*, *Amylase*^{null} *curly wing* survivorship at day 10 increased from 14 $\pm$ 3% when its frequency was .9 to 78 $\pm$ 3% when its frequency decreased to .5, and to 89 $\pm$

4% when its frequency was .1. Note that Amylase^{null} (whether straight or curly wing) survivorship was 0% at day 10 in a monoculture (i.e., its frequency is 1). Figures 9 and 10 clearly show the extent to which Amylase^{null} survivorship is frequency-dependent when competing with the Amylase^{1-a} genotype on the experimental medium (8% starch and 0.5% Brewer's yeast). Furthermore, Amylase^{null} survivorship was found to be frequency-dependent when competing with the Amylase^{1} lobe eye variant (Table 17). An examination of the Amylase^{null} genotypic survivorship in Table 17 shows that its survivorship at day 10 increased from 3 ± 1% to 50 ± 4% to 79 ± 5% when its frequency decreased from .9 to .5 to .1 respectively. Figure 14 helps to illustrate the above statement.

Furthermore, the LT_{10} (the time required till 10% of the flies have died) (Figures 11 and 12) demonstrates that it takes only two days for 10% of the Amylase^{null} flies to die when raised in a monoculture (frequency = 1) on the experimental medium whereas it took approximately eleven days (Figure 11) for 10% of them to die when they were competing with Amylase^{1-a} on the experimental medium.

In the mixed culture experiment, competition for the limited amount of yeast was not observed in a manner that has been defined by Bakker (1961, 1969; Emlen, 1973 and others) that two or more organisms of the same (or different) species exert a disadvantageous influence upon each other; on the contrary, the amylase-producing strains did in fact cause the Amylase^{null}

survivorship to increase significantly. Thus, one could label this phenomenon as a unique case in which the enzymatic by-products of one genotype positively enhance the survivorship of another genotype which lacks the enzymes in question (amylase). Nevertheless this case of intragenotypic interaction is similar to the phenomenon of syntrophism which involves mutual crossfeeding between microbial mutants with different metabolic blocks (Braun, 1966). Furthermore, Bos (1979) has reported that mutual facilitation occurred between two genotypes of Drosophila melanogaster (ebony and F-spa), in which the viability of both genotypes increased significantly when raised in a mixed culture. He attributed this significant increase in viability to the by-products. However, he did not offer any suggestion as to what kind of by-products and at which level they acted.

Amylase and Sugar Excretion by Adults:

Two separate questions arise. The first is why Amylase^{null} survivorship increases significantly pending its frequency when competing for a limited amount of yeast with an amylase-producing strain. The second question is can this phenomenon be observed in Drosophila melanogaster larvae.

I predicted that these results are possible if the amylase-producing strains made sugar available to the Amylase^{null} strain. This can be achieved by either one or both of the following mechanisms. The first is that the amylase-producing strain (e.g. Amylase^{1-a}) consumed an excessive amount of starch and digested it

in the digestive tract, thereby absorbing a large percentage as glucose and excreting a given percentage of it into the medium. Thus, the Amylase^{null} strain would utilize the excreted sugar. If this is true, then one would expect to detect sugar in the medium inhabited by an amylase-producing strain, and to find no sugar in the medium inhabited by Amylase^{null} or inhabited by no flies as a control. The results in Figure 15 do in fact point out that the sugar concentration in the medium (8% starch and 0.5% Brewer's yeast) inhabited by Amylase^{1-a} increases as the number of Amylase^{1-a} males increase, however no increase in the sugar concentration was observed in the control medium (inhabited by no flies). Note that only males were used in this experiment because females lay eggs and eggs have a small amount of amylase activity (Doane, 1967). In such a situation it would not be possible to tell whether the sugar present in the medium is that excreted by the flies or that resulting from external starch digestion by amylase from the Drosophila melanogaster eggs.

In addition, it is clear that the sugar concentration is a function of the number of Amylase^{1-a} individuals (Figure 15). This helps to explain why Amylase^{null} survivorship is frequency-dependent when competing with an amylase-producing strain. For example, when 90 Amylase^{1-a} flies competed with 10 Amylase^{null} flies for the limited amount of yeast, the 90 Amylase^{1-a} flies excreted enough sugar into the experimental medium to sustain over 80% of the Amylase^{null} flies. Now let us look at the opposite proportion where 90 Amylase^{null} flies competed with 10 Amyla^{1-a}

flies. In this case, the sugar excreted by the 10 *Amylase*$^{1-a}$ flies was not sufficient to keep more than approximately 10% of the *Amylase*^{null} flies alive after twelve days of competition.

The second way in which carbohydrates can be made available to the *Amylase*^{null} individuals when competing with an amylase-producing strain is that of external starch digestion, i.e., if the amylase-producing individuals excreted active amylase enzymes into the common medium. The excreted amylase would then break down the amylose chains into dextrins, glucose and maltose; consequently, the *Amylase*^{null} genotype would benefit from this phenomenon. If the amylase-producing strains do in fact excrete active amylase enzymes into the medium, then one should be able to detect it.

The results in Figure 16 show that the amylase-producing strain (*Amylase*$^{1-a}$) did in fact excrete active amylase enzymes into the experimental medium. Furthermore, Figure 18 confirms that the amylase found in the medium comes from the genotype that inhabited the medium. Only males were used to confirm that the amylase-producing strains excreted active amylase enzymes into the medium, as females lay eggs which have amylase activity (Doane, 1967).

It is not possible of course to state that the sugar in the experimental medium is derived from external digestion, i.e., by the excreted amylase, or from the internal digestion of starch (excretion of sugar). However, it is entirely possible to state that the sugar in the medium came about by the combination of the

two mechanisms.

These results may help to explain Ayala's (1969, 1970) results which showed that Drosophila pseudoobscura and Drosophila serrata continued to co-exist, whereas the exclusion principle predicted the elimination of one of the species. One could argue that in spite of the fact that the two species were competing for the same limited resources in the experimental vials, there is no evidence indicating that they were competing for every single component of the medium. Furthermore, these unknown (and unaccounted for) components increase as the flies continuously modify their environment by excreting various enzymes and various by-products into it. Finally, the outcome of Ayala's (1969, 1970) experiments might have been different if the biochemistry of the media were taken into consideration. In my experiment for instance, the Amylase^{null} strain did not survive more than eight days in a monoculture on the experimental medium; however, in a mixed culture, with an amylase-producing strain its survivorship significantly increased and became frequency-dependent simply because the amylase-producing strain modified the environment in favour of the Amylase^{null} genotype.

Now I shall attempt to clarify how amylase enzymes are being excreted into the medium. My results strongly suggest that amylase is being excreted from the posterior end of the digestive tract. Doane (personal communication) pointed out that the acidity of the posterior portion of the gut would destroy the enzyme before it reached the external milieu. However, the counter

argument would be that a high percentage of the amylase enzymes are being engulfed inside the excreted solid material and thus escape the acidity. In humans, salivary amylase remains active in the stomach acidity for more than thirty minutes, as the saliva amylase is engulfed by the bolus and is therefore not immediately destroyed by the stomach acidity (Townes et al, 1976). Furthermore, Townes et al (1976) have clearly demonstrated that duodenal aspirates contained salivary amylase in patients who have lost their pancreatic function, suggesting that this enzyme survived passage through the stomach acidity.

Amylase and Sugar Excretion by Larvae, and their Carbohydrate Requirements:

Now I shall address the second question of whether larvae behave as the Amylase null adults do when raised in a mixed culture on the experimental medium with an amylase-producing strain. Changes in the egg-to-adult viability of Drosophila melanogaster was found to be affected by larval density (Chiang and Hodson, 1950) as well as by the presence of larvae of other genotypes (Budnik and Brncic, 1976; Bos et al, 1977; Bos, 1979, and others). Furthermore, Kojima and Tobari (1967, 1969b) have also shown that the egg-to-adult viability in Drosophila melanogaster at the esterase-6 and at the alcohol dehydrogenase loci is frequency-dependent. They offered a similar explanation, that the metabolic by-products were responsible for the changes in pre-adult viability. Although this explanation is correct, my study is the only one

which pinpoints a specific enzyme (amylase) to be responsible for the observed facilitation in the mixed culture of adult Drosophila melanogaster.

The results presented in Figures 19 and 20 do in fact point out that the larvae modify the medium in which they live. As in the medium inhabited by the adults, the medium inhabited by the larvae was found to contain sugar as well as amylase enzymes. Gel electrophoresis (Figures 21 and 22) demonstrated that this amylase is Drosophila amylase and comes from the genotype inhabiting the medium.

The question of why a genotype, such as $\underline{\text{Amylase}}^{1\text{-a}}$ larvae, excreted more amylase than others, such as $\underline{\text{Amylase}}^{6}$ larvae, even though the larvae of both genotypes have similar amylase activity (Figure 23) led me to postulate that in the $\underline{\text{Amylase}}^{1\text{-a}}$ larvae, amylase activity must be located towards the posterior part of the midgut; thus, the amylase enzymes will pass through a short portion of the digestive tract before being excreted. However, the amylase activity in the $\underline{\text{Amylase}}^{6}$ larvae would be located primarily in the anterior portion of the midgut; consequently, the enzymes would have to journey through most of the digestive tract before they reached the external milieu. During this journey, most of the amylase enzymes would have been broken down by proteolytic enzymes. It is to be noted that the difference in amylase excretion is less dramatic in the F_1 heterozygous larvae ($\underline{\text{Amylase}}^{6}$ / $\underline{\text{Amylase}}^{1\text{-a}}$) (See Figure 23).

An examination of the amylase distribution (Figure 24) along the digestive tracts of the three genotypes (Amylase^{1-a}, Amylase^{6} and Amylase^{1-c}) reveals that the results are mixed and do not constitute a clear cause-effect relationship. Nonetheless, the pattern of amylase distribution agrees with the findings of Abraham and Doane (1978). The differential excretion of amylase by Amylase^{1-a} and Amylase^{6} can be explained by either of, or by the combination of the following two ways: firstly, amylase heat stability, with Amylase^{1-a} being more stable than Amylase^{6}; and secondly, as has been pointed out by Boorn and Scharloo (1978) that starch in combination with yeast induces amylase activity at different rates in different strains of Drosophila melanogaster (see Literature Review, pp.41-42). In other words, starch combined with a low yeast level might have induced the biosynthesis of more Amylase^{1-a} enzymes in the Amylase^{1-a} genotype than Amylase^{6} enzymes in the Amylase^{6} variant.

Lastly, since larvae modify their environment in the same way as the adults do, one would expect the Amylase^{null} larvae to behave in a fashion similar to the Amylase^{null} adults when competing with an amylase-producing strain. In other words, one would predict that the Amylase^{null} larval viability would increase as their frequency decreases when competing with an amylase-producing strain. The results obtained did not agree with the above prediction. This led me to question the larval carbohydrate requirements. The data in Figures 25A, 25B and 25C point out that larvae require more protein than carbohydrates. For example, at a

high yeast level (6%), no added carbohydrate is needed for 50% of the eggs to reach adulthood. Even when the yeast level decreased to 3%, approximately 50% of the eggs again reached adulthood without supplementary carbohydrates (Figure 25B). On the other hand, at a 2% yeast level, a minimum of 1% carbohydrates was required before any egg reached the adult stage (Figure 25C). This clearly indicates that larvae require more protein than sugar. This is because larvae are in their developmental stage and need building material (amino acids) in order to reach adulthood whereas adults require more carbohydrates as a source of energy rather than building material.

Finally, it is possible to observe frequency-dependent viability in *Amylase*^{null} larvae when competing with an amylase-producing strain but a more elaborate and well-known medium composition is required, as the Brewer's yeast used in this experiment contains various components including some carbohydrates.

In summary, I have shown that the *Amylase*^{null} adult survivorship in a monoculture, on a medium poor in Brewer's yeast but rich in starch, reaches 0% within eight days whereas its survivorship is frequency-dependent when competing on the same medium with an amylase-producing strain. This was found to be the consequence of the fact that the amylase-producing strain excretes active amylase enzymes into the common culture medium, thus making carbohydrates available to the *Amylase*^{null} genotype.

Even though the amylase-producing larvae excreted amylase into the experimental medium, the Amylase^{null} larval viability was not frequency-dependent. This is because larval carbohydrate requirements differ significantly from those of the adults.

VII SUMMARY AND CONCLUSION

Monitoring the survivorship of amylase-producing and amylase deficient strains of Drosophila melanogaster on various food media, and the biochemical analysis of the media inhabited by these strains permit one to conclude the following:

1) The survivorship of the amylase-producing and of the amylase deficient strains were similar under the following conditions:

a) on a medium rich in Brewer's yeast regardless of the carbohydrates.

b) on a medium poor in Brewer's yeast but containing glucose.

c) on a medium poor in Brewer's yeast and having no carbohydrates. On this medium, both strains died within eight days.

2) The amylase-producing strain survived significantly better than the amylase deficient strain on a medium poor in Brewer's yeast and rich in starch.

3) The females survived significantly better than the males. This difference was attributed to the variance in body size, the females being larger than the males.

4) Density does affect survivorship, however, this difference was not significant under the densities used in this experiment.

5) The amylase deficient strain survivorship increased significantly when this strain was raised with an amylase-producing strain on a medium poor in Brewer's yeast but rich in starch. Furthermore, the improvement in survivorship of the amylase deficient strain is frequency-dependent.

6) The amylase producing strain excreted active amylase enzymes into the food medium which it inhabited. This was confirmed by a biochemical assay (DNSA assay) and by gel electrophoresis.

7) The excreted amylase has been shown to modify the medium by breaking down the starch into maltose.

8) Larvae also excrete active amylase enzymes into the food medium which they occupy.

9) It has been shown that the larval nutrient requirements differ substantially from that of the adults.

10) This study has shown that selective pressure was acting primarily on the amylase locus, and that the survivorship of the amylase deficient strain was frequency-dependent for which the underlying physiological and biochemical mechanism is fully understood for the first time.

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IX APPENDIX

Table 1: Raw data on the survivorship of *Amy*^{null} and *Amy*^{4,6} on media A and B

Time in Days	*Amy*^{null} survivorship							*Amy*^{4,6} survivorship						
	Replicate number					Mean ± 1 S.E.	% ± 1 S.E.	Replicate number					Mean ± 1 S.E.	% ± 1 S.E.
	1	2	3	4	5			1	2	3	4	5		
Medium A contained 8% Brewer's yeast and 8% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	9	10	9	10	10	9.6±.25	96±2.45	10	9	10	10	10	9.8±.20	98±2.00
4	9	9	9	9	10	9.2±.20	92±2.00	10	9	10	10	10	9.8±.20	98±2.00
6	9	9	9	8	9	8.8±.20	88±2.00	8	9	10	10	10	9.4±.40	94±4.00
8	8	8	8	8	8	8.0±.00	80±0.00	8	9	9	10	8	8.8±.40	88±3.93
10	8	8	7	7	8	7.6±.25	76±2.45	7	9	9	10	8	8.6±.51	86±5.10
Medium B contained 2% Brewer's yeast and 8% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	10	10	9	10	9	9.6±.24	96±2.40	10	10	10	10	10	10	100%
4	9	8	7	9	8	8.2±.36	82±3.64	10	9	10	10	10	9.8±.20	98±2.00
6	5	7	7	9	7	7.0±.62	70±6.15	10	8	10	10	10	9.6±.40	96±4.00
8	5	7	6	8	6	6.4±.50	64±4.96	10	8	10	10	10	9.6±.40	96±4.00
10	5	7	6	7	6	6.2±.37	62±3.73	10	8	10	10	10	9.6±.40	96±4.00

Table 2: Raw data on the survivorship of *Amy*^{null} and *Amy*^{4,6} on media C and D

Time in Days	*Amy*^{null} survivorship							*Amy*^{4,6} survivorship						
	Replicate number					Mean ± 1 S.E.	% ± 1 S.E.	Replicate number					Mean ± 1 S.E.	% ± 1 S.E.
	1	2	3	4	5			1	2	3	4	5		
Medium C contained .5% Brewer's yeast and 8% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	10	8	9	9	8	8.8±.37	88±3.74	10	10	10	10	10	10	100%
4	4	3	5	4	6	4.4±.51	44±5.10	10	10	10	10	10	10	100%
6	2	2	3	2	2	2.2±.20	22±2.00	10	10	10	10	10	10	100%
8	1	0	1	1	0	.6±.24	6±2.45	10	10	10	10	10	10	100%
10	0	0	0	0	0	0	0	10	9	10	10	10	9.8±.20	98±2.00
Medium D contained 8% Brewer's yeast and 2% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
4	10	9	10	9	10	9.6±.24	96±2.45	10	10	10	10	10	10	100%
6	8	9	9	9	8	8.6±.24	86±2.45	10	10	10	10	9	9.8±.20	98±2.00
8	8	8	8	8	7	7.8±.20	78±2.00	9	9	8	9	8	8.6±.24	86±2.45
10	7	7	8	7	7	7.2±.20	72±2.00	9	8	7	8	8	8.0±.32	80±3.16

Table 3: Raw data on the survivorship of *Amy*^{null} and *Amy*^{4,6} on media E and F

Time in Days	*Amy*^{null} survivorship							*Amy*^{4,6} survivorship						
	Replicate number					Mean ±	% ±	Replicate number					Mean ±	% ±
	1	2	3	4	5	1 S.E.	1 S.E.	1	2	3	4	5	1 S.E.	1 S.E.
Medium E contained 2% Brewer's yeast and 2% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	8	9	10	10	9	9.2±.37	92±3.74	10	10	10	10	10	10	100%
4	8	8	9	9	9	8.6±.25	86±2.45	10	10	10	10	10	10	100%
6	8	8	9	9	9	8.6±.25	86±2.45	10	10	10	10	10	10	100%
8	8	7	8	9	8	8.0±.32	80±3.16	10	8	9	10	10	9.4±.40	94±4.00
10	7	6	7	7	8	7.0±.32	70±3.16	10	8	7	8	10	8.6±.60	86±5.99
Medium F contained .5% Brewer's yeast and 2% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	10	9	8	10	9	9.2±.37	92±3.74	10	10	10	10	10	10	100%
4	3	3	4	5	6	4.2±.58	42±5.82	10	9	10	10	10	9.8±.20	98±2.00
6	2	1	3	2	2	2.0±.30	20±3.16	10	9	10	9	9	9.4±.25	94±2.50
8	1	1	2	1	0	1.0±.32	10±3.16	10	9	10	9	9	9.4±.25	94±2.50
10	1	0	1	0	0	.4±.55	4±5.48	9	8	10	8	9	8.8±.37	88±3.74

Table 4: Raw data on the survivorship of *Amy*^{null} and *Amy*^{4,6} on media G and H

Time in Days	*Amy*^{null} survivorship							*Amy*^{4,6} survivorship						
	Replicate number					Mean ±	% ±	Replicate number					Mean ±	% ±
	1	2	3	4	5	1 S.E.	1 S.E.	1	2	3	4	5	1 S.E.	1 S.E.
Medium G contained 8% Brewer's yeast and 0% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	10	10	10	9	10	9.8±.20	98±2.00	10	10	10	10	10	10	100%
4	10	9	9	9	10	9.4±.25	94±2.45	10	10	10	10	10	10	100%
6	8	7	9	8	8	8.0±.32	80±3.16	10	8	10	10	10	9.6±.40	96±4.00
8	7	6	8	8	7	7.2±.37	72±3.74	8	8	8	9	9	8.4±.24	84±2.45
10	6	6	8	7	7	6.8±.37	68±3.74	7	7	9	9	7	7.8±.49	78±4.89
Medium H contained 2% Brewer's yeast and 0% starch														
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	9	10	9	10	10	9.8±.25	98±2.45	10	10	10	10	10	10	100%
4	9	9	8	9	10	9.0±.32	90±3.16	10	10	10	10	10	10	100%
6	9	8	8	8	8	8.2±.20	82±2.00	10	10	10	10	10	10	100%
8	7	8	8	7	7	7.4±.25	74±2.45	9	10	9	9	8	9.0±.32	90±3.16
10	7	6	7	6	7	6.6±.25	66±2.45	9	10	9	8	7	8.6±.51	86±5.10

Table 5A: Raw data on the survivorship of Amy^{null} and Amy^{4,6} on medium I

Medium I contained .5% Brewer's yeast and 0% starch

Time in Days	Amy^{null} survivorship							Amy^{4,6} survivorship						
	Replicate number					Mean ±	% ±	Replicate number					Mean ±	% ±
	1	2	3	4	5	1 S.E.	1 S.E.	1	2	3	4	5	1 S.E.	1 S.E.
0	10	10	10	10	10	10	100%	10	10	10	10	10	10	100%
2	10	9	10	10	10	9.8±.20	98±2.00	10	10	10	10	10	10	100%
4	8	6	7	5	8	6.8±.58	68±5.82	10	8	10	8	8	8.8±.50	88±4.89
6	2	1	3	3	2	2.2±.37	22±3.74	8	6	6	5	7	6.4±.51	64±5.10
8	1	0	0	0	0	.2±.20	2±2.00	2	3	3	2	3	2.6±.25	26±2.45
10	0	0	0	0	0	0	0	0	0	2	1	1	.8±.37	8±3.74

Table 5B: Raw data on the survivorship of Amy^{null} on medium I

Medium I contained .5% Brewer's yeast and 0% starch

Time in Days	Amy^{null} survivorship (replicate number)										Mean ± 1 S.E.	% ± 1 S.E.
	1	2	3	4	5	6	7	8	9	10		
0	100	100	100	100	100	100	100	100	100	100	100	100%
2	80	79	84	80	85	81	83	81	79	78	81.0± .72	81.0± .72
4	44	43	50	50	50	44	51	51	42	48	47.3±1.15	47.3±1.15
6	2	5	4	2	3	7	13	8	6	12	6.2±1.23	6.2±1.23
8	0	0	0	0	0	0	2	0	1	2	.5± .27	.5± .27
10	0	0	0	0	0	0	0	0	0	0	0	0

Table 5C: Raw data on the survivorship of *Amy*$^{1-a}$ and *Amy*^{1}, *lobe eye* on medium I

Medium I contained .5% Brewer's yeast and 0% starch

Time in Days	*Amy*$^{1-a}$ survivorship on medium I (replicate #)										Mean ± 1 S.E.	% ± 1 S.E.
	1	2	3	4	5	6	7	8	9	10		
0	100	100	100	100	100	100	100	100	100	100	100	100%
2	96	90	89	92	95	94	97	95	94	90	93.2± .88	93.2± .88
4	45	60	65	62	57	60	64	59	64	60	59.6±1.81	59.6±1.81
6	15	27	29	28	20	27	26	21	25	26	24.4±1.39	24.4±1.39
8	3	10	14	8	12	7	8	7	6	5	8.0±1.03	8.0±1.03
10	1	5	5	0	2	2	2	1	1	1	2.0± .54	2.0± .54
12	0	1	1	0	0	0	0	0	0	0	.2± .13	.2± .13

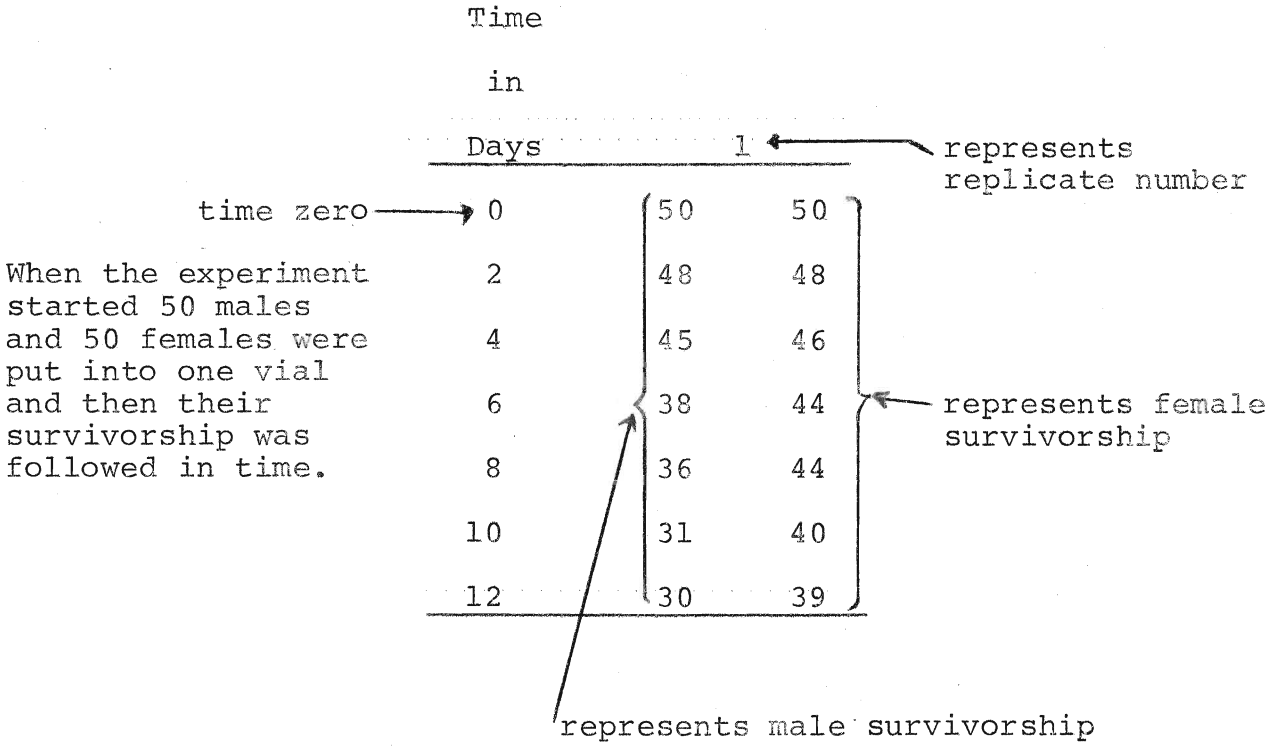
Time in Days	*Amy*^{1}, *lobe eye* survivorship on medium I										Mean ± 1 S.E.	% ± 1 S.E.
	1	2	3	4	5	6	7	8	9	10		
0	100	100	100	100	100	100	100	100	100	100	100	100%
2	85	82	80	69	75	71	73	78	78	82	77.3±1.65	77.3±1.65
4	29	35	29	35	45	28	36	35	39	38	34.9±1.66	34.9±1.66
6	7	3	6	8	8	5	8	6	9	10	7.0± .65	7.0± .65
8	0	3	3	1	6	0	0	0	1	2	1.6± .62	1.6± .62
10	0	0	1	0	0	0	0	0	0	1	.2± .13	.2± .13

Table 6: Raw data representing the survivorship of $\underline{Amy}^{null}$, $\underline{Amy}^{4,6}$ and $\underline{Amy}^{1-a}$ on two media (1- experimental medium made up of 8% starch and 0.5% Brewer's yeast, and 2- control medium made up of 8% glucose and 0.5% Brewer's yeast), and at four different densities (100, 50, 25 and 10 flies per vial, made up of 50% ♂♂ and 50% ♀♀.)

Time in Days	The survivorship of 3 amylase variants on experimental medium (no. 3)											
	null Amy				1-a Amy				4,6 Amy			
0	100	50	25	10	100	50	25	10	100	50	25	10
2	82	45	23	10	100	50	25	10	99	50	25	10
4	30	12	6	5	100	49	25	10	93	46	24	9
6	4	1	1	1	99	49	25	10	92	45	23	8
8	0	0	0	0	98	49	24	10	88	43	22	8
10	0	0	0	0	96	48	24	10	87	42	21	7
	The survivorship of 3 amylase variants on control medium											
0	100	50	25	10	100	50	25	10	100	50	25	10
2	90	50	25	10	98	49	23	10	96	50	24	10
4	90	49	25	10	96	47	23	10	90	47	24	10
6	89	48	25	9	96	47	23	10	88	45	24	10
8	88	48	24	9	94	46	23	10	88	44	23	9
10	87	47	24	9	83	43	23	10	88	44	23	9

Guide for Tables 7A to 11A:

Each table contains ten replicates on starch (upper half of each table) and ten replicates on glucose (lower half of each table). For example, let us look at replicate number 1 of Table 7A.



Guide for Tables 7B to 11B:

The B tables contain the mean ± one standard of error obtained from the A tables; furthermore, these means were converted into percentages ± one standard of error.

Table 7A: Raw data on the survivorship of Amy straight wing adults on two media. In each replicate the initial number was 100 flies per vial. (50 ♂♂ and 50 ♀♀)

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast									
	Replicate Number									
	1	2	3	4	5	6	7	8	9	10
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	48 48	49 50	50 49	49 50	48 50	50 50	49 50	49 49	50 50	48 50
4	45 46	49 46	45 49	48 49	47 50	49 50	47 50	48 49	50 49	48 50
6	38 44	49 44	44 48	47 49	47 50	48 50	47 50	46 48	49 48	48 50
8	36 44	49 44	44 48	46 48	47 50	48 50	47 50	46 48	49 48	48 50
10	31 40	49 44	43 47	46 46	45 49	47 49	47 50	44 48	49 48	46 50
12	30 39	47 42	41 46	44 45	43 48	47 46	46 50	44 48	48 47	46 49
	Control Medium: 8% glucose + 0.5% Brewer's yeast									
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	50 50	50 50	50 50	50 50	50 50	50 50	50 49	50 50	49 50	50 49
4	50 50	50 50	50 49	50 50	49 50	50 50	50 49	50 49	49 50	50 49
6	50 50	48 49	50 49	50 50	49 50	50 50	50 49	49 48	48 50	49 49
8	50 50	45 47	50 49	50 50	49 49	49 49	50 49	49 48	48 50	49 49
10	50 50	43 45	48 48	49 50	48 48	49 49	50 49	48 48	48 50	49 49
12	50 50	42 42	47 47	49 50	47 48	48 47	49 49	47 48	48 50	49 48

Table 7B: The mean survivorship of Amy 1-a straight wing adults on two media; the means were then converted into percentages.

Time in Days	Amy 1-a straight wing			
	Males		Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
Medium Composition: 8% starch; 0.5% Brewer's yeast				
0	50	100%	50	100%
2	49.0±0.26	98.0±0.52	49.6±0.22	99.2±0.44
4	47.6±0.52	95.2±1.04	48.8±0.49	97.6±0.98
6	46.3±1.04	92.6±2.08	48.1±0.74	96.2±1.48
8	46.0±1.21	92.0±2.42	48.0±0.73	96.0±1.46
10	44.7±1.64	89.4±3.28	47.1±0.98	94.2±1.96
12	43.4±1.62	86.8±3.24	46.0±1.06	92.0±2.12
Control Medium: 8% glucose + 0.5% Brewer's yeast				
0	50	100%	50	100%
2	49.9±0.15	99.8±0.30	49.8±0.13	99.6±0.26
4	49.8±0.13	99.6±0.26	49.6±0.15	99.2±0.30
6	49.4±0.27	98.8±0.54	49.3±0.21	98.6±0.42
8	49.0±0.50	98.0±1.00	48.9±0.28	97.8±0.56
10	48.3±0.65	96.6±1.30	48.5±0.45	97.0±0.90
12	47.5±0.69	95.0±1.38	47.8±0.73	95.6±1.46

Table 8A: Raw data on the survivorship of Amy curly wing on two media (starch and glucose) for males and females. In each replicate the initial number was 100 flies per vial (50♂♂ and 50♀♀).

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast									
	Replicate Number									
	1	2	3	4	5	6	7	8	9	10
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	50 50	49 50	49 50	50 49	50 50	48 49	49 49	48 49	50 50	50 50
4	50 50	49 50	49 49	50 49	50 49	48 49	48 49	48 49	48 50	49 50
6	50 49	48 49	49 49	50 49	50 49	48 49	48 49	48 49	46 50	47 49
8	50 48	47 49	49 49	50 49	48 49	46 48	46 48	47 49	44 49	47 49
10	50 47	47 49	49 49	50 49	48 49	46 47	46 48	47 48	43 48	46 49
12	50 47	46 49	49 49	50 49	47 49	44 47	44 48	47 48	43 48	45 49
	Control Medium: 8% glucose + 0.5% Brewer's yeast									
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	50 50	50 50	50 50	50 50	48 50	50 49	50 50	50 50	49 50	49 50
4	49 50	50 50	50 50	50 50	48 50	50 49	50 49	50 50	49 50	49 50
6	49 50	50 50	50 50	50 50	47 49	49 49	50 49	49 49	49 50	49 50
8	49 50	50 50	50 50	49 50	47 49	49 49	50 49	48 47	47 48	49 50
10	48 49	50 50	49 50	49 50	47 49	48 49	50 49	48 46	47 48	47 50
12	48 49	50 49	49 49	49 50	47 48	48 49	50 49	47 46	47 48	46 49

Table 8B: The mean survivorship of Amy^{1-a} curly wing adults on two media; the means were then converted into percentage survivorships.

Time in Days	Amy^{1-a} curly wing			
	Males		Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
Medium Composition: 8% starch; 0.5% Brewer's yeast				
0	50	100%	50	100%
2	49.3±0.27	98.6±0.54	49.6±0.16	99.2±0.32
4	48.9±0.28	97.8±0.56	49.4±0.16	98.8±0.32
6	48.4±0.43	96.8±0.86	49.1±0.10	98.2±0.20
8	47.4±0.60	94.8±1.20	48.7±0.15	97.4±0.30
10	47.1±0.64	94.2±1.28	48.3±0.26	96.6±0.52
12	46.5±0.81	93.0±1.62	48.3±0.26	96.6±0.52
Control Medium: 8% glucose + 0.5% Brewer's yeast				
0	50	100%	50	100%
2	49.6±0.22	99.2±0.44	49.9±0.10	99.8±0.20
4	49.5±0.22	99.0±0.44	49.7±0.15	99.4±0.30
6	49.2±0.29	98.4±0.58	49.6±0.17	99.2±0.34
8	49.0±0.37	98.0±0.74	49.2±0.33	98.4±0.66
10	48.3±0.37	96.6±0.74	49.0±0.40	98.0±0.80
12	48.0±0.40	96.0±0.80	48.6±0.34	97.2±0.68

Table 9A: The survivorship of Amy, lobe eye adults on two media. In each replicate the initial number at day 0 was 100 flies per vial (50♂♂ and 50♀♀).

Time in Days	Replicate Number									
	1	2	3	4	5	6	7	8	9	10
	Experimental Medium: 8% starch + 0.5% Brewer's yeast									
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	47 46	50 50	43 49	48 50	50 50	43 48	50 50	49 49	50 50	50 50
4	40 44	45 48	39 47	48 50	47 50	42 45	48 49	49 49	48 48	49 50
6	35 42	45 48	33 45	47 49	47 49	42 45	47 49	48 49	48 47	49 48
8	34 41	45 47	31 44	47 49	45 49	38 43	47 49	46 47	48 47	49 48
10	24 40	44 46	28 39	40 46	45 47	38 43	47 49	45 47	48 47	49 48
12	22 34	42 44	26 37	35 43	39 43	38 43	46 49	45 47	48 46	48 48
	Control Medium: 8% glucose + 0.5% Brewer's yeast									
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	49 49	50 50	49 50	50 50	50 50	49 49	50 50	50 50	50 50	50 50
4	48 49	49 50	47 48	50 49	50 50	49 49	50 49	48 49	50 50	49 50
6	48 49	48 50	47 48	50 49	50 50	49 49	50 49	48 49	50 49	49 50
8	47 49	48 50	47 48	50 49	50 50	48 49	50 49	48 49	50 49	48 48
10	46 48	48 50	47 48	50 49	50 50	48 49	50 49	48 48	50 49	48 48
12	46 47	47 50	47 48	49 49	49 50	47 48	50 49	48 48	49 49	48 48

Table 9B: The mean ± one standard of error were converted into percentages ± one standard of error for Amy one, lobe eye.

Time in Days	Medium Composition: 8% starch; 0.5% Brewer's yeast			
	Amy[1], lobe eye			
	Males		Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
0	50	100%	50	100%
2	48.0±0.90	96.0±1.80	49.2±0.42	98.4±0.84
4	45.5±1.21	91.0±2.42	48.0±0.67	96.0±1.34
6	44.1±1.80	88.2±3.60	47.1±0.75	94.2±1.50
8	43.0±2.01	86.0±4.02	46.4±0.90	92.8±1.80
10	41.3±2.39	82.6±4.78	45.2±1.06	90.4±2.12
12	38.9±2.85	77.8±5.70	43.4±1.50	86.8±3.00
	Control Medium: 8% glucose + 0.5% Brewer's yeast			
0	50	100%	50	100%
2	49.7±0.15	99.4±0.30	49.8±0.13	99.6±0.26
4	49.0±0.34	98.0±0.64	49.3±0.22	98.6±0.44
6	48.9±0.35	97.8±0.70	49.2±0.20	98.4±0.40
8	48.6±0.40	97.2±0.80	49.2±0.20	98.4±0.40
10	48.5±0.45	97.0±0.90	48.7±0.22	97.4±0.44
12	48.0±0.40	96.0±0.80	48.6±0.31	97.2±0.62

Table 10A: Raw data on the survivorship of Amy^{null} curly wing adults on two media. In each replicate the initial number was 100 flies per vial, made up of 50♂♂ and 50 ♀♀ .

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast									
	Replicate Number									
	1	2	3	4	5	6	7	8	9	10
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	48 50	50 50	50 50	50 50	30 50	49 50	48 48	48 50	48 49	33 40
4	2 23	4 25	3 10	2 20	1 16	2 23	8 19	1 11	1 12	3 13
6	1 4	0 0	0 1	0 2	0 3	0 0	0 2	0 0	0 0	0 2
8	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
10	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
12	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	Control Medium: 8% glucose + 0.5% Brewer's yeast									
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	50 50	50 50	50 50	49 50	50 50	50 50	49 50	48 50	50 50	49 50
4	49 48	50 50	49 50	49 50	49 50	50 49	49 50	48 50	50 50	49 49
6	47 48	50 50	49 50	49 50	48 50	50 49	49 50	48 49	49 50	48 49
8	44 46	50 49	49 49	49 49	48 49	50 49	49 50	48 49	49 50	48 49
10	43 46	50 49	49 49	48 49	47 49	48 49	49 50	46 48	47 49	48 49
12	43 46	50 49	49 49	48 49	47 48	48 48	49 50	46 48	47 48	48 49

Table 10B: The mean (+ one standard of error) survivorship of Amy^{null} curly wing on two media (starch and glucose) was converted into percentages.

Time in Days	Medium Composition: 8% starch; 0.5% Brewer's yeast			
	Amy^{null} curly wing			
	Males		Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
0	50	100%	50	100%
2	45.5±2.35	91.0±4.70	48.7±0.99	97.4±1.98
4	2.7±0.67	5.4±1.34	17.2±1.75	34.4±3.50
6	0.1±0.10	0.2±0.20	1.4±0.45	2.8±0.90
8	-	-	0.1±0.10	0.2±0.20
10	-	-	-	-
12	-	-	-	-
	Control Medium: 8% glucose + 0.5% Brewer's yeast			
0	50	100%	50	100%
2	49.5±0.23	99.0±0.46	50.0±0.00	100.0±0.00
4	49.1±0.18	98.2±0.36	49.6±0.22	99.2±0.44
6	48.7±0.30	97.4±0.60	49.5±0.23	99.0±0.46
8	48.4±0.54	96.8±1.08	48.9±0.35	97.8±0.70
10	47.5±0.62	95.0±1.24	48.7±0.34	97.4±0.68
12	47.5±0.62	95.0±1.24	48.4±0.34	96.8±0.68

Table 11A: Raw data on the survivorship of Amy^{null} straight wing adults on the experimental and control media. A total of ten replicates were done; the density was 100 flies per vial, made up of 50♂♂ and 50♀♀ at time zero.

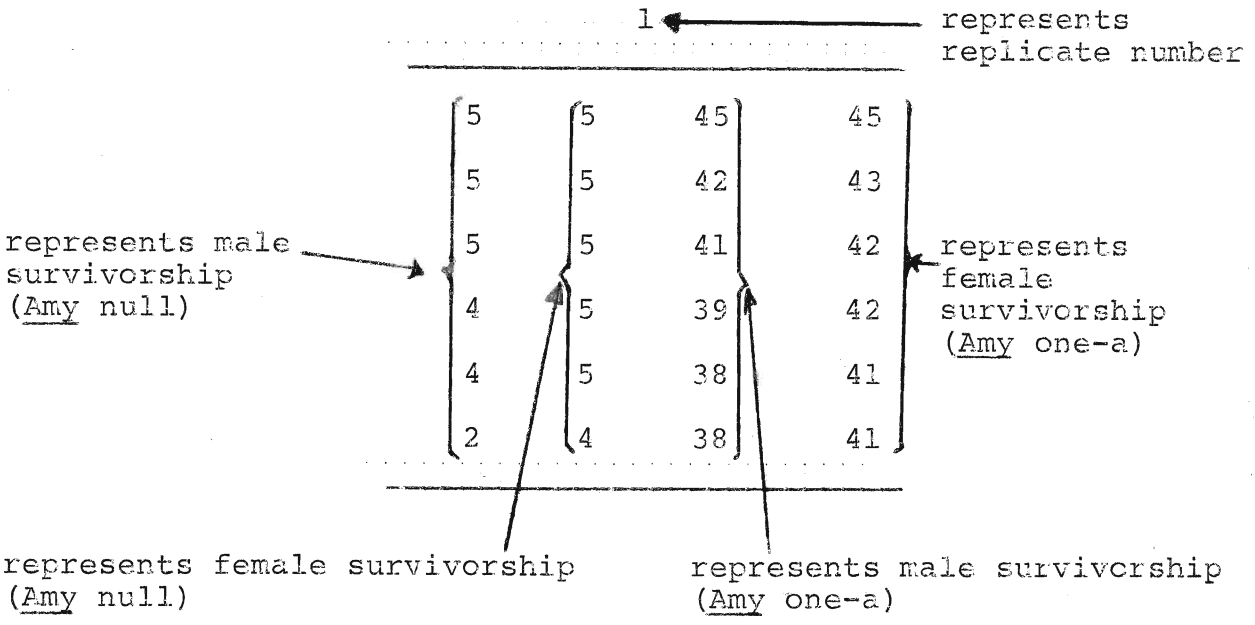
Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast									
	Replicate Number									
	1	2	3	4	5	6	7	8	9	10
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	48 50	49 48	48 50	48 49	48 50	46 50	49 50	48 49	48 50	38 40
4	7 38	5 35	5 34	6 30	3 33	6 32	4 28	2 30	2 29	2 10
6	0 5	0 4	0 4	0 2	0 3	0 0	0 2	0 1	0 1	0 2
8	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
10	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
12	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	Control Medium: 8% glucose + 0.5% Brewer's yeast									
0	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50	50 50
2	50 50	50 50	50 50	50 50	50 50	50 49	49 50	50 50	50 50	50 50
4	50 50	50 50	49 50	50 50	50 50	49 49	49 50	50 50	50 50	50 50
6	50 50	50 50	45 47	50 50	50 50	49 49	49 50	49 50	49 50	50 49
8	50 50	49 49	42 46	49 50	50 50	49 49	49 50	49 50	49 50	50 49
10	50 50	49 49	41 46	48 50	50 50	49 49	49 50	48 50	49 50	50 49
12	50 50	47 47	40 44	47 50	49 50	49 49	49 50	48 49	49 50	50 48

Table 11B: The mean survivorship for Amy^{null} straight wing adults on two food media; the means were converted into percentages.

Time in Days	Males		Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
	Medium Composition: 8% starch; 0.5% Brewer's yeast			
	Amy^{null} straight wing			
0	50	100%	50	100%
2	47.0±0.98	94.0±1.96	48.6±0.98	97.2±1.96
4	4.2±1.03	8.4±2.06	29.9±2.41	59.8±4.82
6	-	-	2.4±0.50	4.8±1.00
8	-	-	0.1±0.10	0.2±0.20
10	-	-	-	-
12	-	-	-	-
	Control Medium: 8% glucose + 0.5% Brewer's yeast			
0	50	100%	50	100%
2	49.9±0.10	99.8±0.20	49.9±0.10	99.8±0.20
4	49.7±0.15	99.4±0.30	49.9±0.10	99.8±0.20
6	49.1±0.47	98.2±0.94	49.6±0.31	99.2±0.62
8	48.6±0.74	97.2±1.48	49.4±0.40	98.8±0.80
10	48.3±0.85	96.6±1.70	49.4±0.40	98.8±0.80
12	47.8±0.93	95.6±1.86	48.8±0.63	97.6±1.26

Guide for Tables 12A to 23A:

Each table contains seven replicates on starch (upper half of each table) and seven replicates on glucose (control) (lower half of each table). For example, let us look at replicate number 1 of Table 12A.



At time zero, there were 100 flies in replicate number 1 made up of ten Amylase$^{null}_{1-a}$ straight wing (5 males and 5 females) and ninety Amylase curly wing (45 males and 45 females).

Guide for Tables 12B to 23B:

Each B table contains the means ± one standard of error for the corresponding A table. These means were converted into percentages ± one standard of error.

Table 12A: The survivorship of both *Amy*^{null} and *Amy*^{la} in time, when 10 *Amy*^{null} *straight wing* (*st.w*) competed with 90 *Amy*^{la} *curly wing* (*cu.w*) on two media.

Time in Days	Medium Composition: 8% starch + 0.5% Brewer's yeast						
	Replicate Number						
	1	2	3	4	5	6	7
0	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45
2	5 5 45 45	5 5 44 45	5 5 44 45	5 5 45 45	5 5 45 45	5 5 44 45	5 5 43 45
4	5 5 42 43	5 5 44 45	5 5 44 45	5 5 45 45	5 5 45 45	5 5 44 45	5 5 43 45
6	5 5 41 42	5 5 43 44	5 5 44 45	5 5 45 44	5 5 44 44	5 5 43 44	5 5 42 45
8	4 5 39 42	4 5 41 42	4 5 40 41	5 5 44 44	5 5 44 43	5 5 43 42	5 5 42 43
10	4 5 38 41	4 5 40 42	4 4 40 41	5 5 44 44	5 5 44 41	5 5 43 42	5 5 42 43
12	2 4 38 41	2 3 32 42	4 4 39 40	5 5 43 44	5 5 44 41	5 5 43 42	5 5 42 43
	Control: Medium Composition: 8% glucose + 0.5% Brewer's yeast						
0	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45
2	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 44	5 5 45 45	5 5 45 45	5 5 44 45
4	5 5 44 45	5 5 45 45	5 5 45 45	5 5 45 44	5 5 45 45	5 5 45 45	5 5 44 45
6	5 5 44 44	5 5 44 45	5 5 45 44	5 5 45 44	5 5 44 44	5 5 44 45	5 5 44 44
8	5 5 44 44	5 5 42 45	5 5 44 45	5 5 45 44	5 5 42 43	5 5 44 45	5 5 43 44
10	5 5 43 44	5 5 42 45	5 5 43 45	5 5 44 44	5 5 42 43	5 5 44 45	5 5 43 44
12	5 5 43 44	5 5 42 45	5 5 43 44	5 5 44 44	4 5 40 43	5 5 41 44	4 4 43 44

Table 12B: The mean and percentage survivorship of *Amy*^{null} and *Amy*$^{1\text{-}a}$ on two food media.

Time in Days	*Amy*^{null} s.w., Sex and Frequency + *Amy*$^{1\text{-}a}$ c.w., Sex and Frequency							
	5 Males		5 Females		45 Males		45 Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
Medium Composition: 8% starch + 0.5% Brewer's yeast								
0	5.0±0.00	100±0.00	5.0±0.00	100±0.00	45.0±0.00	100.0±0.00	45.0±0.00	100.0±0.00
2	5.0±0.00	100±0.00	5.0±0.00	100±0.00	44.3±0.24	98.4±0.54	45.0±0.00	100.0±0.00
4	5.0±0.00	100±0.00	5.0±0.00	100±0.00	43.9±0.34	97.6±0.75	44.7±0.24	99.3±0.54
6	5.0±0.00	100±0.00	5.0±0.00	100±0.00	43.1±0.43	95.8±0.95	43.9±0.29	97.6±0.63
8	4.6±0.17	92±3.40	4.9±0.12	98±2.40	41.9±0.62	93.1±1.37	42.4±0.31	94.2±0.69
10	4.6±0.17	92±3.40	4.9±0.12	98±2.40	41.6±0.73	92.4±1.62	42.0±0.37	93.3±0.82
12	4.0±0.45	80±9.00	4.4±0.25	88±5.00	40.1±1.34	89.1±2.97	41.9±0.43	93.1±0.95
Control: Medium Composition: 8% glucose + 0.5% Brewer's yeast								
0	5.0±0.00	100±0.00	5.0±0.00	100±0.00	45.0±0.00	100.0±0.00	45.0±0.00	100.0±0.00
2	5.0±0.00	100±0.00	5.0±0.00	100±0.00	44.9±0.12	99.8±0.27	44.9±0.12	99.8±0.27
4	5.0±0.00	100±0.00	5.0±0.00	100±0.00	44.7±0.16	99.3±0.35	44.7±0.16	99.3±0.35
6	5.0±0.00	100±0.00	5.0±0.00	100±0.00	44.4±0.17	98.7±0.38	44.4±0.17	98.7±0.38
8	5.0±0.00	100±0.00	5.0±0.00	100±0.00	43.4±0.36	96.4±0.79	44.3±0.24	98.4±0.54
10	5.0±0.00	100±0.00	5.0±0.00	100±0.00	43.0±0.26	95.6±0.58	44.3±0.24	98.4±0.54
12	4.9±0.12	98±2.41	5.0±0.00	100±0.00	42.7±0.44	94.9±0.97	44.0±0.18	97.8±0.41

Table 13A: The survivorship of both *Amy^{null} st.w.* and *Amy^{1-a} cu.w.* in time, when 50 *Amy^{null}* competed with 50 *Amy^{1-a}* on two food media.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast																			
	Replicate Number																			
	1				2				3				4				5			
0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	25	25	25	25	25	25	24	25	25	25	25	25	25	25	25	25	25	24	25	25
4	24	25	25	25	24	25	24	25	23	24	24	25	25	25	25	25	25	24	25	25
6	24	24	24	25	23	24	24	24	22	24	24	24	23	24	25	25	25	24	25	25
8	24	24	24	25	21	24	23	24	20	22	23	22	23	24	25	25	24	23	24	24
10	23	24	24	24	20	23	23	24	20	20	23	22	23	24	25	25	24	23	24	24
12	22	24	24	24	14	16	23	24	16	17	23	22	17	18	22	25	16	20	22	24

	Control Medium: 8% Glucose + 0.5% Brewer's yeast																			
0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	25	25	25	25	25	25	24	25	25	25	25	25	24	25	25	25	25	25	24	25
4	24	25	25	25	24	25	24	24	24	25	25	24	24	25	25	25	24	25	24	24
6	24	25	24	25	24	25	24	24	24	25	25	24	24	25	25	25	24	25	24	24
8	24	25	23	24	24	25	24	24	24	25	25	24	24	25	25	25	24	25	24	24
10	24	25	23	24	23	25	24	24	24	25	25	24	24	25	24	25	24	25	24	24
12	24	24	23	24	23	25	24	24	24	25	25	24	24	25	24	25	24	25	23	24

Time in Days	Replicate Number																			
	6				7				8				9				10			
0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	24	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
4	23	25	25	24	25	24	25	25	25	25	25	25	25	25	25	25	25	25	25	25
6	21	20	23	24	23	24	22	24	24	24	24	24	24	24	24	24	24	24	24	24
8	20	19	22	24	20	21	21	23	23	24	24	24	24	24	24	24	24	24	24	24
10	17	19	22	24	20	21	20	23	23	24	24	24	24	24	24	24	24	24	24	24
12	15	17	22	24	16	19	20	22	23	24	24	24	24	24	24	24	24	24	24	24

0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	25	25	24	25	24	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
4	25	24	24	25	24	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
6	25	24	24	25	24	24	24	25	25	25	25	25	25	25	25	25	25	25	25	25
8	25	24	24	25	24	24	24	25	25	25	25	25	25	25	25	25	25	25	25	25
10	24	24	24	25	24	24	24	25	25	25	25	25	25	25	25	25	25	25	25	25
12	23	24	24	25	24	24	24	25	25	25	25	25	25	25	25	25	25	25	25	25

Table 13B: The mean and percentage survivorship of *Amy*^{null} and *Amy*$^{1\text{-}a}$ on two food media.

Time in Days	*Amy*^{null} st.w., Sex and Frequency				+ *Amy*$^{1\text{-}a}$ cu.w., Sex and Frequency			
	25 Males		25 Females		25 Males		25 Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
Experimental Medium: 8% starch + 0.5% Brewer's yeast								
0	25.0±.00	100.0±0.00	25.0±.00	100.0±0.00	25.0±.00	100.0±0.00	25.0±.00	100.0±0.00
2	24.9±.12	99.6±0.48	24.9±.12	99.6±0.48	24.9±.12	99.6±0.48	25.0±.00	100.0±0.00
4	24.9±.12	99.6±0.48	24.6±.17	98.4±0.68	24.7±.16	98.8±0.64	24.9±.12	99.6±0.48
6	23.0±.41	92.0±1.64	23.4±.41	93.6±1.64	24.3±.16	97.2±0.64	24.3±.16	97.2±0.64
8	21.7±.60	86.8±2.40	22.4±.60	89.6±2.40	23.1±.43	92.4±1.72	23.9±.34	95.6±1.36
10	21.0±.78	84.0±3.12	22.0±.63	88.0±2.52	23.0±.52	92.0±2.08	23.7±.30	94.8±1.20
12	16.6±.80	66.4±3.20	18.7±.85	74.8±3.40	22.3±.40	89.2±1.60	23.6±.36	94.4±1.44
Control Medium: 8% Glucose + 0.5% Brewer's yeast								
0	25.0±.00	100.0±0.00	25.0±.00	100.0±0.00	25.0±.00	100.0±0.00	25.0±.00	100.0±0.00
2	24.7±.16	98.8±0.64	25.0±.00	100.0±0.00	24.7±.16	98.8±0.68	24.9±.12	99.6±0.48
4	24.1±.12	96.4±0.48	24.9±.12	99.6±0.48	24.6±.17	98.8±0.68	24.6±.17	98.4±0.68
6	24.1±.12	96.4±0.48	24.9±.12	99.6±0.48	24.3±.16	97.2±0.64	24.6±.17	98.4±0.68
8	24.1±.12	96.4±0.48	24.7±.16	98.8±0.64	24.1±.12	96.4±0.48	24.5±.17	98.0±0.68
10	23.9±.12	95.6±0.48	24.7±.16	98.8±0.64	24.0±.18	96.0±0.72	24.4±.17	97.6±0.68
12	23.7±.16	94.8±0.64	24.6±.17	98.4±0.68	23.9±.21	95.6±0.84	24.4±.17	97.6±0.68

Table 14A: The survivorship of both *Amy*^{null} and *Amy*$^{1\text{-}a}$ when 90 *Amy*^{null} *st.w.* competed with 10 *Amy* one-a *cu.w.* on two media.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast						
	Replicate Number						
	1	2	3	4	5	6	7
0	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5
2	45 45 5 5	36 44 5 5	45 42 5 5	41 44 5 5	45 45 5 5	44 45 5 5	45 44 5 5
4	38 40 5 5	18 31 5 5	22 28 5 5	30 38 5 5	18 26 5 5	26 35 5 5	29 37 5 5
6	23 25 5 5	15 23 4 5	12 13 5 5	13 23 5 5	0 10 5 5	2 15 5 5	3 18 5 5
8	15 25 5 5	4 12 4 4	3 10 5 5	1 3 4 5	0 7 5 5	2 9 5 5	3 14 5 5
10	9 15 5 5	4 7 4 4	3 10 5 5	1 3 3 5	0 3 5 5	1 8 5 5	2 11 5 5
12	6 14 5 5	1 4 4 4	2 5 5 5	0 2 3 5	0 3 5 5	1 4 5 5	0 7 5 5
	Control Medium: 8% Glucose + 0.5% Brewer's yeast						
0	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5
2	44 45 5 5	45 44 5 5	45 45 5 5	44 45 5 5	45 45 5 5	45 45 5 5	44 45 5 5
4	43 45 5 5	44 43 5 5	44 43 5 5	44 45 5 5	44 44 5 5	45 44 5 5	44 45 5 5
6	42 42 5 5	44 43 5 5	44 43 5 5	44 45 5 5	44 44 5 5	45 44 5 5	44 45 5 5
8	42 42 5 5	42 43 5 5	44 43 5 5	44 45 5 5	44 44 5 5	45 41 5 5	44 45 5 5
10	42 42 5 5	42 43 5 5	44 43 5 5	44 45 5 5	44 43 5 5	45 41 5 5	44 44 5 5
12	41 42 5 5	42 43 5 5	44 43 5 5	44 45 5 5	44 43 5 5	44 41 5 5	44 44 5 5

Table 14B: The mean and percentage survivorship on two media for *Amy*^{null} *straight wing* and *Amy*$^{l\text{-}a}$ *curly wing*.

Time in Days	*Amy*^{null} *s.w.* Sex & Frequency				*Amy*$^{l\text{-}a}$ *c.w.* Sex & Frequency			
	45 Males + 45 Females				5 Males		5 Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
	Medium Composition: 8% starch + 0.5% Brewer's yeast							
0	45%	100%	45%	100%	5%	100%	5%	100%
2	43.0±1.10	95.6±2.41	44.1±0.34	98.0±0.75	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
4	25.9±2.29	57.6±5.08	33.6±1.68	74.7±3.74	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
6	9.7±2.65	21.6±5.88	18.1±1.81	40.2±4.03	4.7±.16	94.0±3.20	5.0±0.0	100.0±0.0
8	4.0±1.59	8.9±3.53	11.4±2.20	25.3±4.90	4.6±.25	92.0±5.00	4.9±.12	98.0±2.40
10	2.9±0.96	6.4±2.12	8.1±1.37	18.0±3.04	4.6±.25	92.0±5.00	4.9±.12	98.0±2.40
12	1.4±0.68	3.1±1.51	5.6±1.28	12.4±2.84	4.4±.25	88.0±5.00	4.7±.16	94.0±3.10
	Control: Medium Composition: 8% glucose + 0.5% Brewer's yeast							
0	45%	100%	45%	100%	5%	100%	5%	100%
2	44.6±0.18	99.1±0.40	44.9±0.12	99.8±0.27	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
4	44.0±0.18	97.8±0.40	44.1±0.29	98.0±0.63	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
6	43.9±0.29	97.6±0.63	43.7±0.35	97.1±0.78	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
8	43.9±0.36	97.6±0.80	43.3±0.48	96.2±1.06	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
10	43.6±0.36	96.9±0.80	43.0±0.41	95.6±0.91	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
12	43.3±0.41	96.2±0.91	43.0±0.41	95.6±0.91	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0

Table 15A: The survivorship of both *Amy*^{null} and *Amy*$^{1\text{-}a}$ when 10 *Amy*^{null} *cu.w.* competed with 90 *Amy*$^{1\text{-}a}$ *st.w.*

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast						
	Replicate Number						
	1	2	3	4	5	6	7
0	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45
2	5 5 43 45	5 5 44 45	4 5 44 45	5 5 44 45	5 5 45 45	5 5 45 45	5 5 45 45
4	5 5 36 45	5 5 44 45	4 5 44 45	5 5 44 45	5 5 45 45	5 5 45 45	5 5 44 44
6	5 5 35 43	5 5 43 44	4 5 43 45	5 5 43 44	5 5 43 43	5 5 44 44	5 5 43 44
8	4 5 35 42	5 5 42 44	4 5 42 45	5 5 43 44	5 5 40 40	4 5 42 43	5 5 42 43
10	4 5 31 37	5 5 41 43	4 4 42 45	4 4 43 43	5 5 39 38	4 5 42 43	4 4 42 43
12	4 4 30 32	3 4 37 39	4 4 41 43	4 4 42 42	5 5 39 38	3 5 41 44	4 4 41 42
	Control Medium: 8% Glucose + 0.5% Brewer's yeast						
0	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45
2	4 5 44 45	5 5 45 45	5 5 43 45	5 5 45 44	5 5 45 45	5 5 45 45	5 5 45 45
4	4 5 43 45	5 5 43 45	5 5 43 45	5 5 45 44	5 5 43 45	5 5 45 45	5 5 45 45
6	4 5 42 43	5 5 43 45	5 5 43 45	5 5 45 44	5 5 42 44	5 5 43 43	5 5 43 45
8	4 5 42 43	5 5 43 45	5 5 43 45	5 5 45 44	5 5 41 44	5 5 42 43	5 5 43 45
10	4 5 41 42	5 5 43 45	5 5 43 45	5 5 45 44	4 5 41 44	4 5 41 43	5 5 43 45
12	4 5 40 38	5 5 42 45	5 5 40 44	5 5 44 44	4 5 40 42	4 5 41 41	5 5 43 45

Table 15B: Mean survivorship ± one standard of error which were converted into percentages ± one standard of error.

Time in Days	null Amy cu.w., Sex and Frequency				+ 1-a Amy st.w., Sex and Frequency			
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
Experimental Medium: 8% starch + 0.5% Brewer's yeast								
0	5.0±0.00	100.0±0.00	5.0±0.00	100.0±0.00	45.0±0.00	100.0±0.00	45.0±0.00	100.0±0.00
2	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	44.3±0.29	98.4±0.65	45.0±0.00	100.0±0.00
4	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	43.1±1.20	95.8±2.70	44.9±0.14	99.8±0.31
6	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	42.0±1.17	93.3±2.54	43.9±0.14	97.6±0.30
8	4.6±0.20	92.0±4.00	4.9±0.14	98.0±2.80	40.9±1.03	90.9±2.30	43.0±0.62	95.6±1,40
10	4.3±0.19	86.0±3.80	4.6±0.20	92.0±4.00	40.0±1.57	88.9±3.50	41.7±1.13	92.7±2.50
12	3.9±0.25	78.0±5.20	4.3±0.19	86.0±3.80	38.7±1.58	86.0±3.5-	40.0±1.56	88.9±3.50
Control Medium: 8% Glucose + 0.5% Brewer's yeast								
0	5.0±0.00	100.0±0.00	5.0±0.00	100.0±0.00	45.0±0.00	100.0±0.00	45.0±0.00	100.0±0.00
2	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	44.6±0.30	99.1±0.70	44.9±0.14	99.7±0.30
4	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	43.7±0.34	97.1±0.80	44.9±0.14	99.7±0.30
6	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	43.1±0.41	95.8±0.90	44.1±0.34	98.0±0.80
8	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	42.7±0.47	94.9±1.10	44.1±0.34	98.0±0.80
10	4.9±0.14	98.0±2.80	5.0±0.00	100.0±0.00	42.4±0.21	94.2±0.50	44.1±0.34	98.0±0.80
12	4.6±0.20	92.0±4.00	5.0±0.00	100.0±0.00	41.7±0.78	92.7±1.70	42.7±0.97	94.9±2.20

Table 16A: The survivorship of both *Amy*^{null} and *Amy*$^{1\text{-}a}$ when 50 *Amy*^{null} *cu.w.* competed with 50 *Amy* one-a *st.w.* on two food media.

Time in Days	Replicate Number																			
	1				2				3				4				5			
Experimental Medium: 8% starch + 0.5% Brewer's yeast																				
0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	24	25	25
4	24	25	25	25	24	25	25	24	25	24	25	25	24	25	25	25	25	22	25	25
6	23	25	25	25	24	24	23	24	24	24	25	25	24	25	25	25	22	21	24	25
8	22	24	25	25	24	23	23	24	23	24	24	25	21	22	25	25	20	18	23	23
10	21	23	24	24	17	18	22	23	21	20	24	25	18	21	24	25	19	18	23	23
12	20	21	24	23	15	17	20	22	16	18	24	23	14	21	21	23	19	18	23	23
Control Medium: 8% Glucose + 0.5% Brewer's yeast																				
0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	24	25	25	24	25	25	25	25	25	24	25	24	25	25	24	25	25	25	25	24
4	23	25	25	24	25	25	25	25	25	24	25	24	25	25	24	25	25	24	25	24
6	23	25	25	24	25	25	25	25	25	24	25	24	25	25	24	25	25	23	25	24
8	23	25	25	24	25	25	24	25	25	24	25	24	25	25	24	25	25	23	25	24
10	23	25	25	24	25	25	24	25	25	24	25	24	25	25	24	25	25	22	25	23
12	22	24	24	24	25	25	24	25	25	23	25	24	25	25	24	24	25	18	23	23

Time in Days	Replicate Number															
	6				7											
Experimental Medium: 8% starch + 0.5% Brewer's yeast																
0	25	25	25	25	25	25	25	25								
2	25	25	25	25	25	25	25	25								
4	25	25	25	25	24	25	24	25								
6	22	21	24	25	24	25	25	25	20	22	23	24				
8	20	18	23	23	21	23	25	24	19	21	22	22				
10	19	18	23	23	20	19	25	24	18	18	22	22				
12	19	18	23	23	15	18	21	21	15	17	18	22				
Control Medium: 8% Glucose + 0.5% Brewer's yeast																
0	25	25	25	25	25	25	25	25								
2	25	25	25	24	24	25	25	25	25	24	25	25				
4	25	24	25	24	24	24	25	25	25	24	25	25				
6	25	23	25	24	25	23	25	24	25	24	25	24				
8	24	23	25	24	24	23	25	24	25	24	25	24				
10	25	22	25	23	24	23	25	24	25	24	24	24				
12	25	18	23	23	24	23	21	23	24	24	23	24				

Table 16B: The mean and percentage survivorship on two food media for Amy null cu.w. and Amy one-a, st.w..

Time in Days	null Amy cu.w., Sex and Frequency: 25 Males Mean + 1 S.E.	25 Males % + 1 S.E.	25 Females Mean + 1 S.E.	25 Females % + 1 S.E.	+ 1-a Amy st.w., Sex and Frequency: 25 Males Mean + 1 S.E.	25 Males % + 1 S.E.	25 Females Mean + 1 S.E.	25 Females % + 1 S.E.
Experimental Medium: 8% starch + 0.5% Brewer's yeast								
0	25.0+.00	100.0+0.00	25.0+.00	100.0+0.00	25.0+.00	100.0+0.00	25.0+.00	100.0+0.00
2	25.0+.00	100.0+0.00	24.9+.14	99.6+0.56	25.0+.00	100.0+0.00	25.0+.00	100.0+0.00
4	24.3+.19	97.2+0.76	24.6+.43	98.4+1.72	24.6+.30	98.4+1.20	24.9+.14	99.6+0.56
6	23.0+.58	92.0+2.32	23.9+.63	95.6+2.52	24.6+.36	98.4+1.44	24.9+.30	99.6+0.56
8	21.1+.51	84.4+2.04	22.1+.80	88.4+3.20	23.4+.43	93.6+1.72	24.0+.44	96.0+1.76
10	19.1+.59	76.4+2.36	20.0+.69	80.0+2.76	23.1+.63	92.4+2.52	23.7+.42	94.8+1.68
12	16.3+.86	65.2+3.44	18.6+.89	74.4+3.56	21.0+.70	84.0+2.80	22.0+.62	88.0+2.48
Control Medium: 8% Glucose + 0.5% Brewer's yeast								
0	25.0+.00	100.0+0.00	25.0+.00	100.0+0.00	25.0+.00	100.0+0.00	25.0+.00	100.0+0.00
2	24.7+.19	98.9+0.76	24.7+.19	98.9+0.76	24.9+.14	99.6+0.56	24.6+.20	98.4+0.80
4	24.6+.15	98.4+0.60	24.4+.82	97.6+3.28	24.9+.14	99.6+0.56	24.4+.20	97.6+0.80
6	24.6+.15	98.4+0.60	24.1+.26	96.4+1.04	24.9+.14	99.6+0.56	24.3+.21	97.2+0.84
8	24.6+.29	98.5+1.16	24.1+.26	96.4+1.04	24.7+.82	98.8+3.28	24.3+.21	97.2+0.84
10	24.6+.29	98.4+1.16	24.0+.43	96.0+1.72	24.6+.82	98.4+3.28	24.1+.26	96.4+1.04
12	24.4+.30	97.6+1.20	23.1+.91	92.4+3.64	23.4+.50	93.6+2.00	23.9+.26	95.6+1.04

Table 17A: The survivorship of both *Amy*^{null} and *Amy*$^{1\text{-}a}$ when 90 *Amy*^{null} *cu.w.* competed with 10 *Amy* one-a *st.w.* on two food media.

Time in Days	Experimental Medium; 8% starch + 0.5% Brewer's yeast						
	Replicate Number						
	1	2	3	4	5	6	7
0	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5
2	45 45 5 5	43 45 5 5	34 44 5 5	40 45 5 5	43 43 5 5	45 45 5 5	44 44 5 5
4	19 31 5 5	29 35 5 5	11 37 5 5	27 37 5 5	13 31 5 5	24 26 5 5	26 34 5 5
6	10 23 5 5	9 20 5 5	9 32 5 5	22 34 5 5	0 9 5 5	0 7 4 5	8 25 5 5
8	5 15 5 5	2 12 5 5	4 24 5 5	12 28 5 5	0 5 4 5	0 5 4 5	4 18 5 5
10	3 11 5 5	1 7 5 5	1 15 5 5	3 21 5 5	0 3 4 5	0 5 4 4	4 14 5 5
12	1 8 5 5	0 6 5 4	1 12 4 5	1 12 5 4	0 3 4 5	0 4 4 4	1 11 5 5
	Control Medium: 8% Glucose + 0.5% Brewer's yeast						
0	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5
2	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	44 45 5 5	45 44 5 5	45 45 5 5
4	45 45 5 5	44 45 5 5	45 45 5 5	45 45 5 5	43 43 5 5	44 44 5 5	45 44 5 5
6	45 44 5 5	44 45 5 5	45 45 5 5	45 45 5 5	42 43 5 5	42 42 5 5	44 44 5 5
8	45 44 5 5	44 45 5 5	45 45 5 5	45 45 5 5	42 43 5 5	41 42 5 5	43 44 5 5
10	45 44 5 5	44 45 5 5	44 45 5 5	45 45 5 5	42 43 4 5	41 42 5 5	43 44 5 5
12	45 44 5 5	44 45 5 5	44 44 5 5	44 45 5 5	41 42 4 5	41 42 5 5	43 43 5 5

Table 17B: The mean survivorship of both Amy^{null} cu.w. and Amy^{1-a} st.w. ± one standard of error; these values were converted into percentages ± one standard of error.

Time in Days	Medium Composition: 8% starch + 0.5% Brewer's yeast							
	Amy^{null} c.w. Sex & Frequency				Amy^{1-a} s.w. Sex & Frequency			
	45 Males		45 Females		5 Males		5 Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
0	45%	100%	45%	100%	5%	100%	5%	100%
2	42.0±1.48	93.3±3.29	44.4±0.30	98.7±0.67	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
4	21.3±2.68	47.3±5.96	33.0±1.49	73.3±3.31	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
6	8.3±2.80	18.4±6.22	21.4±3.93	47.6±8.70	4.9±.14	98.0±2.80	5.0±0.0	100.0±0.0
8	3.9±1.55	8.7±3.44	15.3±3.33	34.0±7.40	4.7±.18	94.0±3.60	5.0±0.0	100.0±0.0
10	1.7±0.60	3.8±1.33	10.9±2.39	24.2±5.31	4.7±.18	94.0±3.60	4.9±.14	98.0±2.80
12	.6±0.20	1.3±0.44	8.0±1.43	17.8±3.18	4.6±.20	92.0±4.00	4.4±.14	88.0±2.80
	Control: Medium Composition: 8% glucose + 0.5% Brewer's yeast							
0	45%	100%	45%	100%	5%	100%	5%	100%
2	44.9±0.14	99.8±0.31	44.9±0.14	99.8±0.31	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
4	44.4±0.15	98.7±0.33	44.4±0.15	98.7±0.33	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
6	43.9±0.51	97.6±1.13	44.0±0.43	97.8±0.96	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
8	43.6±0.61	96.9±1.36	44.0±0.43	97.8±0.96	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0
10	43.4±0.60	96.4±1.33	44.0±0.43	97.8±0.96	4.9±.14	98.0±2.80	5.0±0.0	100.0±0.0
12	43.1±0.59	95.8±1.31	43.7±0.44	97.1±0.98	4.9±.14	98.0±2.80	5.0±0.0	100.0±0.0

Table 18: The survivorship of both Amy^{null} st.w. and Amy^{1-a} st.w. in time, when 10 Amy^{null} st.w. competed with 90 Amy^{1-a} st.w. on two media.

Experimental: Medium Composition: 8% starch; 0.5% Brewer's yeast

Time in Days	Replicate No. 1				Replicate No. 2				null Amy s.w. Sex & Freq. 5 Males + 5 Females M.± 1 SE	null Amy s.w. Sex & Freq. 5 Males + 5 Females % ± 1 SE	null Amy s.w. Sex & Freq. 5 Males + 5 Females M.± 1 SE	null Amy s.w. Sex & Freq. 5 Males + 5 Females % ± 1 SE	1-a Amy s.w. Sex & Freq. 45 Males + 45 Females M.± 1 SE	1-a Amy s.w. Sex & Freq. 45 Males + 45 Females % ± 1 SE	1-a Amy s.w. Sex & Freq. 45 Males + 45 Females M.± 1 SE	1-a Amy s.w. Sex & Freq. 45 Males + 45 Females % ± 1 SE
0	5	5	45	45	5	5	45	45	5%	100%	5%	100%	45%	100%	45%	100%
2	4	5	45	45	5	5	45	45	4.5±.50	90±10.0	5.0±0.00	100±0.00	45.0±0.00	100.0±0.00	45.0±0.00	100.0±0.00
4	4	5	44	45	5	5	45	45	4.5±.50	90±10.0	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	45.0±0.00	100.0±0.00
6	4	5	44	44	5	5	45	45	4.5±.50	90±10.0	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	45.0±0.00	100.0±0.00
8	4	5	42	43	5	5	44	44	4.5±.50	90±10.0	4.5±0.50	90±10.00	42.5±0.50	98.9±1.10	43.5±0.50	96.7±1.11
10	4	5	42	43	5	4	43	41	4.5±.50	90±10.0	4.5±0.50	90±10.00	42.5±0.50	98.9±1.10	42.0±0.50	93.3±1.11
12	4	5	42	43	3	3	39	37	3.5±.50	70±10.0	4.0±1.00	80±20.00	40.5±1.50	90.0±3.33	40.0±3.01	88.9±6.69

Control: Medium Composition: 8% glucose; 0.5% Brewer's yeast

0	5	5	45	45	5	5	45	45	5%	100%	5%	100%	45%	100%	45%	100%
2	5	5	45	45	5	5	44	45	5.0±0.0	100±0.0	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	45.0±0.00	100.0±0.00
4	5	5	45	44	5	5	44	45	5.0±0.0	100±0.0	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	44.5±0.50	98.9±1.10
6	5	5	45	44	5	5	44	45	5.0±0.0	100±0.0	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	44.5±0.50	98.9±1.10
8	5	5	45	44	5	5	44	45	5.0±0.0	100±0.0	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	44.5±0.50	98.9±1.10
10	4	5	45	44	5	5	44	45	4.5±.50	90±10.0	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	44.5±0.50	98.9±1.10
12	4	5	45	44	4	5	44	45	4.0±.57	80±11.4	5.0±0.00	100±0.00	44.5±0.50	98.9±1.10	44.5±0.50	98.9±1.10

where M.± 1 SE = 1 standard of error, and % ± 1 SE = 1 standard of error

Table 19: The survivorship of both *Amy*^{null} and *Amy*$^{1\text{-}a}$ in time, when 50 *Amy*^{null} *st.w.* competed with 50 *Amy*$^{1\text{-}a}$ *st.w.* on two media.

Experimental: Medium Composition: 8% starch; 0.5% Brewer's yeast

Time in Days	Replicate No. 1				Replicate No. 2				*Amy*^{null} *s.w.* Sex & Freq. 25 Males M.± 1 SE	*Amy*^{null} *s.w.* Sex & Freq. 25 Males % ± 1 SE	*Amy*^{null} *s.w.* Sex & Freq. 25 Females M.± 1 SE	*Amy*^{null} *s.w.* Sex & Freq. 25 Females % ± 1 SE	+	*Amy*$^{1\text{-}a}$ *s.w.* Sex & Freq. 25 Males M.± 1 SE	*Amy*$^{1\text{-}a}$ *s.w.* Sex & Freq. 25 Males % ± 1 SE	*Amy*$^{1\text{-}a}$ *s.w.* Sex & Freq. 25 Females M.± 1 SE	*Amy*$^{1\text{-}a}$ *s.w.* Sex & Freq. 25 Females % ± 1 SE
0	25	25	25	25	25	25	25	25	25%	100%	25%	100%		25%	100%	25%	100%
2	24	24	25	25	25	24	25	25	24.5±0.5	98±2.0	24.0±0.0	96±0.0		25.0±0.0	100±0.0	25.0±0.0	100±0.0
4	24	24	25	25	25	24	25	25	24.5±0.5	98±2.0	24.0±0.0	96±0.0		25.0±0.0	100±0.0	25.0±0.0	100±0.0
6	24	24	24	25	23	23	24	25	23.5±0.5	94±2.0	23.5±0.5	94±2.0		24.0±0.0	96±0.0	25.0±0.0	100±0.0
8	24	24	23	25	23	23	24	25	23.5±0.5	94±2.0	23.5±0.5	94±2.0		23.5±0.5	94±2.0	25.0±0.0	100±0.0
10	24	23	23	25	18	20	23	24	21.0±3.0	84±12	21.5±1.5	86±6.0		23.0±0.0	92±0.0	24.5±0.5	98±2.0
12	23	23	23	24	16	17	22	21	19.5±3.5	78±14	20.0±3.0	80±12		22.5±0.5	90±2.0	22.5±0.5	90±2.0

Control: Medium Composition: 8% glucose; 0.5% Brewer's yeast

0	25	25	25	25	25	25	25	25	25%	100%	25%	100%		25%	100%	25%	100%
2	25	25	25	24	25	25	25	25	25±0.0	100±0.0	25.0±0.0	100±0.0		25.0±0.0	100±0.0	24.5±.5	98±2.0
4	25	25	25	24	25	25	24	25	25±0.0	100±0.0	25.0±0.0	100±0.0		24.5±0.5	98±2.0	24.5±.5	98±2.0
6	25	25	25	24	25	24	24	25	25±0.0	100±0.0	24.5±0.5	98±2.0		24.5±0.5	98±2.0	24.5±.5	98±2.0
8	25	24	24	24	25	24	24	25	25±0.0	100±0.0	24.0±0.0	96±0.0		24.0±0.0	96±0.0	24.5±.5	98±2.0
10	25	24	24	24	25	24	24	25	25±0.0	100±0.0	24.0±0.0	96±0.0		24.0±0.0	96±0.0	24.5±.5	98±2.0
12	25	24	24	24	25	23	24	25	25±0.0	100±0.0	23.5±0.5	94±2.0		24.0±0.0	96±0.0	24.5±.5	98±2.0

where M.± 1 SE = 1 standard of error, and % ± 1 SE = 1 standard of error

Table 20: The survivorship of both Amy^{null} st.w. and Amy^{1-a} st.w., when 90 Amy^{null} st.w. competed with 10 Amy^{1-a} st.w. on two media.

Experimental: Medium Composition: 8% starch; 0.5% Brewer's yeast

Time in Days	Replicate No. 1				Replicate No. 2				null Amy s.w. Sex & Freq. 45 Males M.± 1 SE	null Amy s.w. Sex & Freq. 45 Males % ± 1 SE	null Amy s.w. Sex & Freq. 45 Females M.± 1 SE	null Amy s.w. Sex & Freq. 45 Females % ± 1 SE	+	1-a Amy s.w. Sex & Freq. 5 Males M.± 1 SE	1-a Amy s.w. Sex & Freq. 5 Males % ± 1 SE	1-a Amy s.w. Sex & Freq. 5 Females M.± 1 SE	1-a Amy s.w. Sex & Freq. 5 Females % ± 1 SE
0	45	45	5	5	45	45	5	5	45%	100%	45%	100%		5%	100%	5%	100%
2	43	43	5	5	45	45	5	5	44.0±1.0	97.8±2.22	44.0±1.00	97.8±2.22		5.0±0.0	100.0± 0.0	5.0±0.0	100.0± 0.0
4	34	39	5	5	45	43	5	4	30 ±4.01	66.7±8.91	41.0±2.01	91.1±4.47		5.0±0.0	100.0± 0.0	4.5±.50	90.0±10.0
6	30	36	5	5	26	25	4	4	21 ±9.06	46.7±20.1	30.5±5.52	67.8±12.27		4.5±.50	90.0±10.0	4.5±.50	90.0±10.0
8	19	30	5	5	12	16	4	4	11.5±7.53	25.6±16.7	23.0±7.02	51.1±15.60		4.5±.50	90.0±10.0	4.5±.50	90.0±10.0
10	10	16	4	5	4	13	4	4	6.0±4.01	13.3±8.91	14.5±1.50	32.2±3.33		4.0±0.0	80.0± 0.0	4.5±.50	90.0±10.0
12	5	15	4	5	2	5	4	4	3.0±2.01	6.7±4.47	10.0±5.02	22.2±11.16		4.0±0.0	80.0± 0.0	4.5±.50	90.0±10.0

Control: Medium Composition: 8% glucose; 0.5% Brewer's yeast

0	45	45	5	5	45	45	5	5	45%	100%	45%	100%		5%	100%	5%	100%
2	45	45	5	5	44	45	5	5	44.5+.50	98.9+1.11	45.0+0.00	100.0+0.0		5.0+0.0	100.0± 0.0	5.0+0.0	100.0± 0.0
4	45	45	5	5	43	45	5	5	44.0±1.0	97.8±2.22	45.0±0.00	100.0±0.0		5.0±0.0	100.0± 0.0	5.0±0.0	100.0± 0.0
6	45	45	5	5	43	45	5	5	44.0±1.0	97.8±2.22	45.0±0.00	100.0±0.0		5.0±0.0	100.0± 0.0	5.0±0.0	100.0± 0.0
8	45	45	5	5	43	45	5	5	44.0±1.0	97.8±2.22	45.0±0.00	100.0±0.0		5.0±0.0	100.0± 0.0	5.0±0.0	100.0± 0.0
10	45	45	5	5	43	45	5	5	44.0±1.0	97.8±2.22	45.0±0.00	100.0±0.0		5.0±0.0	100.0± 0.0	5.0±0.0	100.0± 0.0
12	45	45	5	5	43	44	5	5	44.0±1.0	97.8±2.22	44.5±0.50	98.9±1.11		5.0±0.0	100.0± 0.0	5.0±0.0	100.0± 0.0

where M.± 1 SE = 1 standard of error, and % ± 1 SE = 1 standard of error

Table 21A: The survivorship of both Amy^{null} st.w. and Amy^{1} lobe eye in time when 10 Amy null st.w. competed with 90 Amy one, lobe eye on two media, (starch and glucose).

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast						
	Replicate Number						
	1	2	3	4	5	6	7
0	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45
2	5 5 41 44	5 5 44 45	5 5 44 44	5 5 44 45	5 5 45 45	5 5 43 45	5 5 44 45
4	5 5 41 44	5 5 44 45	4 5 43 44	5 5 44 45	5 5 45 45	5 5 43 43	5 5 43 43
6	5 5 39 43	5 5 43 43	4 5 41 44	5 5 42 43	3 5 44 45	5 5 42 43	5 5 43 42
8	5 5 37 39	5 5 40 41	4 5 41 43	4 4 40 41	3 5 44 45	4 4 42 42	4 5 43 41
10	4 4 36 38	4 5 37 38	4 5 41 43	3 4 39 41	3 5 43 43	3 3 40 40	4 4 42 40
12	4 3 32 38	3 4 29 36	3 4 38 42	3 3 38 41	3 4 43 43	2 3 40 40	3 4 42 40
	Control Medium: 8% Glucose + 0.5% Brewer's yeast						
0	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45	5 5 45 45
2	5 5 44 45	5 5 45 45	5 5 43 45	5 5 45 45	5 5 45 45	5 5 44 45	5 5 45 45
4	5 5 44 44	5 5 45 45	5 5 43 45	5 5 45 45	5 5 44 43	5 5 44 44	5 5 45 45
6	5 5 44 44	5 5 45 45	5 5 43 45	5 5 45 45	5 5 44 43	5 5 43 44	5 5 45 44
8	5 5 44 44	5 5 45 45	5 5 43 45	5 5 44 44	5 5 42 43	5 5 43 44	5 5 45 44
10	5 5 44 44	5 5 45 45	5 5 43 45	5 5 44 44	5 5 42 43	5 5 43 44	5 5 45 44
12	5 5 44 44	5 5 45 45	5 5 43 45	5 5 44 44	5 5 42 43	5 5 43 44	5 5 45 44

Table 21B: The mean and percentage survivorship (± one standard of error) of *Amy*^{null} and *Amy*^{1}, *lobe eye* on two media.

Time in Days	*Amy*^{null} *st.w.* Sex & Frequency				+ *Amy*^{1}, *lobe eye* Sex & Frequency			
	5 Males		5 Females		45 Males		45 Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
	Medium Composition: 8% starch + 0.5% Brewer's yeast							
0	5%	100%	5%	100%	45%	100%	45%	100%
2	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0	43.6±.48	96.9±1.10	44.7±.19	99.3±.42
4	4.9±.14	98.0±2.8	5.0±0.0	100.0±0.0	43.3±.34	96.2±0.76	44.1±.34	98.0±.76
6	4.6±.30	92.0±6.0	5.0±0.0	100.0±0.0	42.0±.62	93.3±1.38	43.3±.36	96.2±.80
8	4.1±.26	82.0±5.2	4.7±.19	94.0±3.8	41.0±.72	91.1±1.60	41.7±.72	92.7±1.60
10	3.6±.20	72.0±4.0	4.3±.29	86.0±5.8	39.7±.80	88.2±1.78	40.6±.79	90.2±1.76
12	3.0±.22	60.0±4.4	3.6±.21	72.0±4.2	37.4±.91	83.1±2.02	40.2±.91	89.3±2.02
	Control: Medium Composition: 8% glucose + 0.5% Brewer's yeast							
0	5%	100%	5%	100%	45%	100%	45%	100%
2	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0	44.4±.30	98.7±0.67	44.9±.14	99.8±.31
4	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0	44.3±.29	98.4±0.64	44.3±.29	98.4±.64
6	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0	44.1±.34	98.0±0.76	44.1±.34	98.0±.76
8	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0	44.1±.27	98.0±0.60	44.1±.42	98.0±.93
10	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0	43.7±.42	97.1±0.93	44.1±.26	98.0±.58
12	5.0±0.0	100.0±0.0	5.0±0.0	100.0±0.0	43.7±.42	97.1±0.93	44.1±.26	98.0±.58

where Mean ± 1 S.E. = 1 standard of error, and % ± 1 S.E. = 1 standard of error

Table 22A: The survivorship of both *Amy*^{null} *st.w.* and *Amy*^{1}, *lobe eye* in time when 50 *Amy null*, *st.w.* competed with 50 *Amy* one, *lobe eye* on two food media.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast																							
	Replicate Number																							
	1				2				3				4				5				6				7			
0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	25	25	25	25	25	25	23	24	24	25	21	24	23	25	20	24	25	25	25	24	24	25	25	25	25	25	25	25
4	25	25	25	25	22	23	23	24	24	25	20	23	22	24	17	23	25	25	25	24	24	25	25	24	24	24	25	25
6	24	24	23	24	22	23	23	24	24	23	20	22	21	24	17	23	15	15	25	24	14	15	25	24	14	14	25	25
8	23	23	23	24	20	21	22	23	17	18	19	22	15	17	17	23	10	13	24	24	13	14	24	23	8	10	24	24
10	13	15	20	23	12	18	20	21	14	16	18	22	12	15	17	23	9	13	24	24	12	12	24	23	7	8	24	24
12	12	13	17	21	10	12	16	20	12	14	17	21	10	11	17	21	4	12	22	24	3	12	19	23	3	8	24	24
	Control Medium: 8% Glucose + 0.5% Brewer's yeast																							
0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	25	25	25	25	25	25	25	25	25	24	25	25	25	25	25	24	25	24	25	25	25	24	25	25	25	25	25	25
4	24	25	25	25	25	25	25	24	25	23	25	25	25	25	25	24	24	24	25	25	25	24	25	25	25	25	25	25
6	24	25	25	25	25	25	25	24	25	23	25	25	25	25	25	24	24	24	25	25	24	24	25	25	25	25	25	25
8	24	25	25	25	25	24	24	24	23	23	24	24	25	24	25	24	25	25	24	24	24	24	25	25	25	25	25	25
10	24	25	25	25	25	24	24	24	23	23	25	25	25	24	25	24	24	23	25	25	24	24	24	25	25	25	25	25
12	24	25	25	25	25	24	24	24	23	23	23	24	24	24	25	24	24	23	25	25	23	24	24	25	25	25	25	25

Table 22B: The mean and percentage survivorship (+ one standard of error) of Amy^{null} st.w. and Amy^{1-a} lobe eye on two food media.

Time in Days	Amy^{null} st.w., Sex and Frequency				+ Amy^{1-a} lobe, Sex and Frequency			
	25 Males		25 Females		25 Males		25 Females	
	Mean + 1 S.E.	% + 1 S.E.	Mean + 1 S.E.	% + 1 S.E.	Mean + 1 S.E.	% + 1 S.E.	Mean + 1 S.E.	% + 1 S.E.
Experimental Medium: 8% starch + 0.5% Brewer's yeast								
0	25.0+0.00	100.0+0.00	25.0+0.00	100.0+0.00	25.0+0.00	100.0+0.00	25.0+0.00	100.0+0.00
2	24.4+0.30	97.6+1.19	25.0+0.00	100.0+0.00	23.4+0.81	93.6+3.25	24.4+0.20	97.6+0.80
4	23.7+0.48	94.8+1.90	24.4+0.29	97.6+1.18	22.9+1.20	91.6+4.82	24.0+0.31	96.0+1.24
6	19.1+1.75	74.4+6.99	19.7+1.79	78.8+7.17	22.6+1.17	90.4+4.68	23.9+0.26	95.6+1.04
8	15.1+1.94	60.4+7.76	16.6+1.73	66.4+6.91	21.9+1.05	87.6+4.21	23.3+0.29	93.2+1.15
10	11.3+0.92	45.2+3.67	13.9+1,22	55.6+4.90	21.0+1.13	84.0+4.53	20.9+0.40	83.6+1.62
12	7.7+1.58	30.8+6.33	11.7+0.71	46.8+2.85	18.9+1.14	75.6+4.56	22.0+0.62	88.0+2.46
Control Medium: 8% Glucose + 0.5% Brewer's yeast								
0	25.0+0.00	100.0+0.00	25.0+0.00	100.0+0.00	25.0+0.00	100.0+0.00	25.0+0.00	100.0+0.00
2	25.0+0.00	100.0+0.00	24.6+0.21	98.4+0.82	24.9+0.14	99.6+0.56	24.9+0.14	99.6+0.56
4	24.7+0.19	98.6+0.74	24.3+0.29	97.2+1.15	24.9+0.14	99.6+0.56	24.7+0.19	98.8+0.74
6	24.6+0.21	98.4+0.82	24.3+0.29	97.2+1.15	24.9+0.14	99.6+0.56	24.7+0.19	98.8+0.74
8	24.3+0.29	97.2+1.15	24.1+0.26	96.4+1.10	24.7+0.19	98.8+0.74	24.7+0.19	98.8+0.74
10	24.3+0.29	97.2+1.15	24.0+0.31	96.0+1.24	24.6+0.20	98.4+0.80	24.7+0.19	98.9+0.74
12	24.0+0.31	96.0+1.22	24.0+0.31	96.0+1.24	24.3+0.29	97.2+1.15	24.5+0.20	98.0+0.82

Table 23A: The survivorship of both *Amy*^{null} *st.w.* and *Amy*^{1}, *lobe eye* in time when 90 *Amy*-null *st.w.* competed with 10 *Amy*-one, *lobe eye* on two media.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast						
	Replicate Number						
	1	2	3	4	5	6	7
0	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5
2	42 45 5 5	44 45 5 5	42 44 4 5	42 45 4 5	45 45 5 5	43 44 5 5	45 45 5 5
4	40 42 5 5	26 32 5 5	21 34 4 5	16 39 4 5	27 35 5 5	37 42 5 5	30 44 4 5
6	3 17 5 4	12 21 5 5	7 14 4 5	2 19 4 5	0 10 5 5	0 13 5 5	0 9 4 5
8	0 6 5 4	2 13 5 5	0 4 3 5	0 1 3 5	0 1 4 4	0 1 5 4	0 0 4 4
10	0 5 5 4	0 4 4 5	0 4 3 5	0 1 3 5	0 1 4 4	0 1 5 4	0 0 4 4
12	0 3 5 3	0 2 4 4	0 2 3 4	0 1 3 5	0 0 2 4	0 1 4 4	0 0 3 4
	Control Medium: 8% Glucose + 0.5% Brewer's yeast						
0	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5
2	45 45 5 5	45 45 5 5	45 45 5 5	45 45 5 5	45 44 5 5	45 45 5 5	44 44 5 5
4	45 45 5 4	45 44 5 5	45 44 5 5	45 45 5 5	45 44 5 5	45 45 5 5	44 44 5 5
6	45 45 5 4	45 44 5 5	45 44 5 5	44 45 5 5	45 44 5 5	44 44 5 5	43 44 5 5
8	45 45 5 4	45 44 5 5	44 44 5 5	44 45 5 5	45 44 5 5	44 44 5 5	43 44 5 5
10	45 44 5 4	45 44 5 5	44 44 5 5	44 45 5 4	44 43 5 5	43 44 5 5	43 44 5 5
12	45 44 5 4	45 44 5 5	43 44 5 5	44 45 5 4	44 43 5 5	43 44 5 5	43 44 5 5

Table 23B: The mean and percentage survivorship (± one standard of error) for both *Amy*^{null} *st.w.* and *Amy*^{1}, *lobe eye* on two media.

Time in Days	*Amy*^{null} *st.w.* Sex & Frequency				+	*Amy*^{1}, *lobe eye* Sex & Frequency			
	45 Males		45 Females			5 Males		5 Females	
	Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.		Mean ± 1 S.E.	% ± 1 S.E.	Mean ± 1 S.E.	% ± 1 S.E.
	Medium Composition: 8% starch + 0.5% Brewer's yeast								
0	45%	100%	45%	100%		5%	100%	5%	100%
2	43.3±.52	96.2±1.16	44.7±.19	99.3±0.42		4.7±0.19	94.0±3.80	5.0±0.0	100±0.00
4	28.1±3.18	62.4±7.10	38.3±1.76	85.1±3.91		4.6±0.20	92.0±4.00	5.0±0.0	100±0.00
6	3.4±1.71	7.6±3.80	14.7±1.69	32.7±3.76		4.4±0.20	88.0±4.00	4.9±.14	98±2.80
8	0.3±0.30	0.7±0.70	3.9±1.71	8.7±3.80		4.0±0.31	80.0±6.20	4.4±.21	88±4.20
10	-	-	2.3±0.75	5.1±1.67		4.0±0.31	80.0±6.20	4.4±.21	88±4.20
12	-	-	1.3±0.42	2.9±0.93		3.6±0.43	72.0±8.60	4.0±.22	80±4.40
	Control: Medium Composition: 8% glucose + 0.5% Brewer's yeast								
0	45%	100%	45%	100%		5%	100%	5%	100%
2	44.9±.14	99.8±0.31	44.7±.19	99.3±0.42		5.0±0.00	100.0±0.0	5.0±0.0	100±0.00
4	44.9±.14	99.8±0.31	44.4±.20	98.7±0.44		5.0±0.00	100.0±0.0	4.9±.14	98±2.80
6	44.6±.30	99.1±0.67	44.4±.20	98.7±0.44		5.0±0.00	100.0±0.0	4.9±.14	98±2.80
8	44.3±.19	98.4±0.42	44.3±.19	98.4±0.42		5.0±0.00	100.0±0.0	4.9±.14	98±2.80
10	44.0±.31	97.8±0.69	44.1±.22	98.0±0.49		5.0±0.00	100.0±0.0	4.7±.19	94±3.80
12	43.9±.34	97.6±0.76	44.0±.22	97.8±0.49		5.0±0.00	100.0±0.0	4.7±.19	94±3.80

where Mean ± 1 S.E. = 1 standard of error, and % ± 1 S.E. = 1 standard of error

Table 24: Transformation of *Amy*^{null} mean percentage survivorship into Arcsin (from Table 14A in the results section, p.78) when *Amy*^{null} competed with *Amy*$^{1-a}$ *c.w.*

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast							
	Amy^{null} *st. w.* Frequency				Statistical Comparison			
	.1	.5	.9	1.0	.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	90	90	90	90	NS	NS	NS	NS
2	90.00±0.00	86.37± 3.97	79.69± 7.27	86.37± 8.13	NS	NS	NS	2.245
4	90.00±0.00	84.26± 4.37	54.45±12.11	35.73±12.11	1.724	7.374	7.664	19.157
6	90.00±0.00	74.44± 7.27	33.77±12.92	8.91± 4.05	4.390	11.849	13.932	195.200
8	77.08±9.81	69.91± 8.91	24.43±11.83	1.81± 1.81	1.806	14.646	15.214	32.705
10	77.08±9.81	68.03± 9.63	20.44± 9.28	-	2.225	19.309	21.332	32.759
12	66.42±15.3	57.17±10.47	16.22± 8.52	-	1.732	15.878	10.393	11.999

NS = not significant, $P \geq 0.05$; * = $P < 0.05 = 1.985$; ** = $P < 0.01 = 2.627$; *** = $P < 0.001 = 3.393$

d.f. = 98

Table 25: Transformation of Amy^{null} cu.w. mean percentage survivorship into Arcsin (from Table 15 in the results section, p.81) when Amy^{null} cu.w. competed with Amy^{1-a} st.w.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast							
	Amy^{null} st.w. Frequency				Statistical Comparison			
	.1	.5	.9	1.0	.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	90	90	90	90	NS	NS	NS	NS
2	84.26± 6.80	87.44± [illegible]	78.46± 8.13	76.06±10.47	NS	NS	NS	1.325
4	84.26± 6.80	81.47± 6.29	50.94±12.39	26.49± 8.91	.642	7.8[illegible]	7.985	28.293
6	81.87± 6.80	75.58± 8.91	35.06±15.89	7.04± 4.25	1.502	7.[illegible]2	8.564	64.155
8	77.08±10.63	68.36± 9.28	27.56±13.44	1.81± 1.81	2.004	10.797	11.503	27.899
10	70.63±11.39	62.17± 9.28	21.97±10.47	-	2.315	15.314	14.643	[illegible]821
12	64.90±12.25	56.66±10.78	18.05± 7.71	-	2.140	15.278	14.927	18.222

NS = not significant, $P \geq 0.05$; * = $P < 0.05 = 1.985$; ** = $P < 0.01 = 2.627$; *** = $P < 0.001 = 3.393$

d.f. = 98

Table 26: Transformation of Amy^{null} st.w. mean percentage survivorship into Arcsin (from Table 16 in the results section, p.84) when Amy^{null} st.w. competed with Amy^{1-a} st.w.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast							
	Amy^{null} st.w. Frequency				Statistical Comparison			
	.1	.5	.9	1.0	.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	90	90	90	90	NS	NS	NS	NS
2	90	80.02± [illegible]	61.47± 8.53	86.37± 8.13	NS	NS	NS	.711
4	77.08±12.92	80.02± 5.74	62.65±15.00	35.73±12.11	.392	2.676	1.928	5.090
6	77.08±12.92	75.82± 8.13	49.20±23.73	8.91± 4.05	.186	2.248	2.224	17.659
8	71.56±18.44	75.82± 8.13	38.29±23.73	1.81± 1.81	.392	3.412	2.714	8.990
10	71.56±18.44	67.21±17.46	28.52±14.31	-	.371	5.706	5.732	9.000
12	60.00±22.79	62.72±21.13	21.39±16.22	-	.201	4.321	3.647	5.000

NS = not significant, $P \geq 0.05$; * = $P < 0.05 = 1.985$; ** = $P < 0.01 = 2.627$; *** = $P < 0.001 = 3.393$

d.f. = 98

Table 27: Transformation of *Amy*^{null} *st.w.* mean percentage survivorship into Arcsin (from Table 17 in the results section, p.85) when *Amy*^{null} *st.w.* competed with *Amy*^{1}, *lobe eye*.

Time in Days	Experimental Medium: 8% starch + 0.5% Brewer's yeast							
	Amy^{null} *st.w.* Frequency				Statistical Comparison			
	.1	.5	.9	1.0	.1 vs .5	.5 vs .9	.1 vs .9	.1 vs 1.0
0	90	90	90	90	NS	NS	NS	NS
2	90	83.71± 4.44	81.47± 4.97	86.37± 8.13	NS	NS	NS	2.245
4	84.26± 6.80	78.76± 7.04	59.21±13.56	35.73±12.11	1.345	3.915	4.433	17.475
6	78.46± 9.98	61.07±15.45	26.56±11.21	8.91± 4.05	2.517	6.999	15.689	30.775
8	69.63±12.25	52.77±15.68	12.52± 8.72	1.81± 1.81	2.857	7.646	16.557	19.529
10	62.72±12.79	45.23±11.97	9.28± 5.26	-	4.392	10.935	15.368	16.122
12	54.33±11.97	38.59±12.39	6.80± 3.93	-	4.325	8.106	14.934	15.349

NS = not significant
$P \geq 0.05$

	d.f. =	58	98	108	138	148	188
* = $P < 0.05$	=	2.002	1.985	1.982	1.977	1.976	1.973
** = $P < 0.01$	=	2.663	2.627	2.622	2.612	2.610	2.602
*** = $P < 0.001$	=	3.467	3.393	3.383	3.363	3.358	3.343

Table 28A: Maltose concentration (in a medium containing 8% starch and 0.5% Brewer's yeast) relative to the number of males inhabiting the medium.

Replicate Number	Male Genotype and Numbers					Medium only (no flies were present)
	1-a 100 *Amy*	1-a 90 *Amy*	1-a 50 *Amy*	1-a 10 *Amy*	null 100 *Amy*	
	Abs. at t=0	Abs. at t=0	Abs. at t=0	Abs. at t=0	Abs. at t=0	Abs. at t=0
1	.179	.183	.100	.050	.030	.030
2	.125	.180	.130	.060	.020	.030
3	.250	.250	.140	.040	.010	.020
4	.260	.260	.150	.030	.020	.010
5	.400	.350	.170	.060	.010	.030
6	.360	.260	.140	.040	.010	.020
7	.390	.260	.180	.040	.030	.020
8	.300	.270	.180	.050	.010	.020
9	.380	.350	.140	.040	.030	.030
10	.310	.250	.160	.050	.010	.010
11	.240	.230	.130	.060	.020	.010
12	.300	.260	.170	.040	.010	.020
M. ± 1 S.E.	.291 ± .019	.259 ± .015	.149 ± .007	.047 ± .003	.018 ± .003	.021 ± .003

Table 28B: Absorption values converted into maltose concentrations per ml of a medium inhabited by different numbers of flies per vial (100, 90, 50 and 10).

Male Genotype	Number of Males per Vial	Absorbance in nm.	Maltose Concentration per 1 ml	Absorbance in nm.	Maltose Produced per Male per 48 hrs
-	nil	.021 ± .003	.550 ± .045	-	-
null *Amy*	100	.018 ± .003	.530 ± .045	.005 ± .001	.046 ± .043
1-a *Amy*	100	.291 ± .019	2.080 ± .054	.087 ± .006	.092 ± .046
1-a *Amy*	90	.259 ± .015	1.900 ± .051	.086 ± .006	.092 ± .046
1-a *Amy*	50	.149 ± .007	1.270 ± .047	.089 ± .009	.093 ± .048
1-a *Amy*	10	.047 ± .003	.700 ± .045	.141 ± .009	.123 ± .048

Table 29A: Maltose concentration in the control medium containing 8% starch and 0.5% Brewer's yeast. No flies were raised on this medium.

Replicate Number	0 hrs.			12 hrs.			24 hrs.			48 hrs.		
	t_0	t_{30}	ΔD.O.	t_0	t_{30}	ΔD.O.	t_0	t_{30}	ΔD.O.	t_0	t_{30}	ΔD.O.
1	.040	.050	.010	.030	.040	.010	.020	.040	.020	.030	.040	.010
2	.040	.060	.020	.040	.060	.020	.020	.030	.010	.040	.040	.000
3	.050	.060	.010	.040	.050	.010	.030	.040	.010	.050	.070	.020
4	.040	.060	.020	.050	.050	.000	.040	.040	.000	.040	.050	.010
5	.060	.070	.010	.030	.040	.010	.030	.050	.020	.030	.040	.010
6	.030	.050	.020	.020	.030	.010	.020	.030	.010	.040	.050	.020
Mean	.043	.058	.015	.035	.045	.010	.027	.038	.012	.038	.050	.012
$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
1 S.E.	.005	.003	.003	.005	.005	.003	.003	.003	.003	.003	.005	.003

Table 29B: Absorption representing amylase activity in a medium (0.5% Brewer's yeast and 8% starch) inhabited by Amy^{1-a} males for various periods of time.

Replicate	3 hours			6 hours			12 hours			24 hours			48 hours		
Number	t_0	t_{30}	Δ D.O.	t_0	t_{30}	Δ D.O.	t_0	t_{30}	Δ D.O.	t_0	t_{30}	Δ D.O.	t_0	t_{30}	Δ D.O.
1	.030	.050	.020	.040	.100	.060	.050	.100	.050	.070	.120	.050	.100	.180	.080
2	.020	.040	.020	.040	.100	.060	.050	.100	.050	.060	.090	.030	.100	.180	.080
3	.030	.040	.010	.020	.060	.040	.060	.080	.020	.090	.120	.030	.100	.190	.090
4	.040	.060	.020	.020	.030	.010	.060	.090	.030	.090	.110	.020	.100	.220	.120
5	.020	.040	.020	.030	.050	.020	.040	.070	.030	.070	.100	.030	.130	.150	.020
6	.020	.030	.010	.030	.040	.010	.050	.080	.030	.050	.090	.040	.110	.200	.090
7	.010	.040	.030	.020	.040	.020	.050	.090	.040	.060	.080	.020	.120	.210	.090
8	.010	.030	.020	.020	.060	.040	.040	.080	.040	.050	.100	.050	.090	.210	.120
9	.030	.050	.020	.030	.050	.020	.030	.070	.040	.040	.080	.040	.090	.190	.100
10	.030	.030	.000	.030	.040	.010	.030	.050	.020	.030	.090	.050	.100	.180	.080
11	.040	.050	.020	.040	.060	.020	.040	.060	.020	.080	.120	.040	.120	.150	.030
12	.020	.040	.020	.030	.090	.060	.040	.080	.040	.060	.080	.020	.130	.210	.080
Mean + 1 S.E.	.024 + .004	.042 + .003	.018 + .002	.029 + .002	.060 + .007	.031 + .006	.045 + .003	.079 + .004	.034 + .003	.062 + .006	.098 + .005	.035 + .003	.108 + .004	.189 + .007	.082 + .009

Table 30: Values of Amylase activity obtained from Amy^{1-a} males raised on the standard food medium for various periods of time. Readings were taken at 550 nm.

Replicate Number	0 hr (6-8 day old flies)			12 hours			24 hours			48 hours		
	Absorption t_0	Absorption t_{30}	Δ O.D.	Absorption t_0	Absorption t_{30}	Δ O.D.	Absorption t_0	Absorption t_{30}	Δ O.D.	Absorption t_0	Absorption t_{30}	Δ O.D.
1	0	.260	.260	0	.280	.280	0	.260	.260	0	.280	.280
2	0	.300	.300	0	.290	.290	0	.280	.280	0	.240	.240
3	0	.280	.280	0	.300	.300	0	.310	.310	0	.290	.290
4	0	.300	.300	0	.280	.280	0	.290	.290	0	.270	.270
5	0	.320	.320	0	.330	.330	0	.310	.310	0	.260	.260
6	0	.300	.300	0	.340	.340	0	.310	.310	0	.340	.340
7	0	.310	.310	0	.360	.360	0	.320	.320	0	.290	.290
8	0	.310	.310	0	.320	.320	0	.300	.300	0	.350	.350
9	0	.370	.370	0	.360	.360	0	.330	.330	0	.330	.330
10	0	.380	.380	0	.320	.320	0	.310	.310	0	.310	.310
11	0	.310	.310	0	.310	.310	0	.320	.320	0	.310	.310
12	0	.280	.280	0	.300	.300	0	.340	.340	0	.290	.290
Mean $\pm$ 1 S.E.	0	.310$\pm$.010	.310 $\pm$ .010	0	.316$\pm$.010	.316 $\pm$ .010	0	.307$\pm$.010	.307 $\pm$ .010	0	.297$\pm$.010	.297 $\pm$ .010

Table 31: Values of amylase activity obtained from Amy^{1-a} males raised on a medium containing 0.5% Brewer's yeast and 8% starch for various periods of time.

Replicate Number	3 hours		6 hours		12 hours		24 hours		48 hours	
	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}
1	0	.240	0	.310	0	.280	0	.300	0	.290
2	0	.280	0	.310	0	.310	0	.300	0	.210
3	0	.260	0	.300	0	.310	0	.320	0	.210
4	0	.340	0	.280	0	.280	0	.250	0	.250
5	0	.410	0	.460	0	.260	0	.230	0	.300
6	0	.380	0	.450	0	.350	0	.270	0	.290
7	0	.360	0	.410	0	.320	0	.310	0	.260
8	0	.250	0	.380	0	.310	0	.280	0	.280
9	0	.290	0	.300	0	.350	0	.270	0	.200
10	0	.340	0	.300	0	.330	0	.310	0	.210
11	0	.350	0	.360	0	.330	0	.280	0	.250
12	0	.340	0	.340	0	.360	0	.320	0	.220
Mean ± 1 S.E.	0	.320±.016	0	.350±.018	0	.316±.010	0	.287±.010	0	.248±.011

Table 32 : Amylase activity in $\underline{Amy}^{1-a}$ Males raised on a food medium rich in starch(8%) and rich in B. Yeast (5%)for different period of times.

Replicate	3hours		6 houres		]2 houres		24 houres		48 houres	
Number	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}
1	0	.320	0	.300	0	.320	0	.31	0	.32
2	0	.340	0	.300	0	.340	0	.300	0	.300
3	0	.260	0	.320	0	.300	0	.300	0	320
4	0	.340	0	.310	0	.310	0	.310	0	.330
5	0	.250	0	.310	0	.280	0	.410	0	.38
6	0	.360	0	.360	0	.290	0	.320	0	.390
7	0	.380	0	.400	0	.330	0	.300	0	.410
8	0	.400	0	.380	0	.350	0	.350	0	.380
9	0	.300	0	.380	0	.320	0	.340	0	.320
10	0	.270	0	.350	0	.320	0	.320	0	.320
11	0	.330	0	.370	0	.360	0	.340	0	.340
12	0	.310	0	.350	0	.350	0	.320	0	.35
Mean $\pm$ 1.S.E.	0 $\pm$ 0	.322 $\pm$ .014	0 $\pm$ 0	.344 $\pm$ .010	0 $\pm$ 0	.321 $\pm$ .010	0 $\pm$ 0	.327 $\pm$ .010	0 $\pm$ 0	.347 $\pm$ .010

Table 33: Amylase activity in food media inhabited by third instar Amy^{null} larvae. No larvae were allowed to inhabit the control food medium.

Time in Hrs.	Control Medium with no Larvae			Replicate Number 1			2			3		
	t 0	t 60	ΔO.D.	t 0	t 60	ΔO.D.	t 0	t 60	ΔO.D.	t 0	t 60	ΔO.D.
				5% starch in Tris Borate buffer pH 7.5								
0	.080	.110	.030	0.090	0.120	.030	.100	.115	.015	0.090	0.120	.030
2	.002	.012	.010	0.950	0.950	.000	.301	.294	.000	0.661	0.538	.000
4	.010	.030	.020	0.350	0.320	.000	.501	.500	.000	0.650	0.560	.010
8	.001	.051	.050	0.000	0.002	.002	.000	.003	.003	0.001	0.003	.002
16	.005	.035	.030	1.500	1.500	.000	.750	.750	.010	1.500	1.500	.000
24	.020	.040	.020	0.850	0.852	.002	.941	.942	.001	0.820	0.825	.005
				10% Sucrose in Tris Borate buffer pH 7.5								
0	.040	.048	.008	0.050	0.055	.005	.041	.050	.009	0.098	0.120	.022
2	.035	.038	.003	0.060	0.004	.000	.060	.040	.000	0.102	0.060	.000
4	.020	.026	.006	0.115	0.065	.000	.046	.068	.002	0.090	0.070	.000
8	.018	.023	.005	0.001	0.045	.041	.040	.018	.000	0.000	0.040	.004
16	.028	.032	.004	0.139	0.140	.001	.000	.050	.050	2.000	2.000	.000
24	.040	.042	.002	0.098	0.100	.002	.800	.800	.000	1.400	1.400	.020

Table 34: Amylase activity in food media inhabited by third instar Amy^{1-a} larvae. No larvae were allowed into the control food medium.

Time in Hrs.	Control Medium with no Larvae			Replicate Number 1			2			3		
	t_0	t_{60}	ΔO.D.	t_0	t_{60}	ΔO.D.	t_0	t_{60}	ΔO.D.	t_0	t_{60}	ΔO.D.
	5% starch in Tris Borate buffer pH 7.5											
0	.002	.052	.050	.005	0.065	0.060	0.008	0.082	0.074	.003	0.035	0.032
2	.004	.084	.080	.061	0.116	0.055	0.073	0.120	0.047	.186	0.292	0.106
4	.003	.029	.026	.090	0.155	0.065	0.202	0.315	0.113	.270	0.330	0.060
8	.004	.081	.077	.327	0.450	0.123	0.410	0.530	0.120	.323	0.470	0.147
16	.002	.082	.080	.295	0.510	0.215	0.316	0.495	0.179	.335	0.470	0.135
24	.009	.080	.071	.356	0.545	0.189	0.350	0.540	0.190	.412	0.545	0.133
	10% Sucrose in Tris Borate buffer pH 7.5											
0	.017	.025	.008	.040	0.068	0.028	0.085	0.105	0.020	.090	0.112	0.022
2	.107	.111	.004	.125	0.615	0.490	0.079	0.620	0.541	.055	0.483	0.428
4	.032	.065	.033	.076	0.900	0.824	0.175	0.640	0.465	.108	0.535	0.427
8	.035	.040	.005	.205	2.0000	1.795	0.280	2.000	1.720	.298	2.000	1.702
16	.040	.055	.015	.373	1.500	1.127	1.130	2.000	0.870	.615	1.900	1.285
24	.045	.065	.020	.480	1.600	1.120	1.500	2.000	0.500	.950	1.850	0.900

Table 35: Amylase activity in both the third instar (*Amy*^{null} and *Amy*$^{1-a}$) larvae and in the food medium they inhabited for various periods of time. No larvae were raised on the control medium. The values were used in order to plot Figures 19 and 20.

Time in	Control Medium with no Larvae			*Amy*^{null} (amylase activity)				*Amy*$^{1-a}$ (amylase activity)			
				In Medium (excretion)			In Larvae	In Medium (excretion)			In Larvae
	t 0	t 60	ΔO.D. ± 1 S.D.	t 0	t 60	ΔO.D. ± 1 S.D.	ΔO.D. ± 1 S.D.	t 0	t 60	ΔO.D. ± 1 S.D.	ΔO.D. ± 1 S.D.
	5% starch in Tris Borate buffer pH 8.7										
0	.050	.081	.031±.021	.060	.085	.025±.010	.016±.010	.005	0.061	0.056±.024	0.730±.070
2	.003	.048	.045±.040	.008	.007	.000±.000	.012±.003	.106	0.176	0.070±.036	0.792±.013
4	.007	.037	.030±.010	.021	.018	.000±.000	.011±.010	.187	0.267	0.080±.020	1.718±.022
8	.003	.066	.063±.018	.000	.003	.003±.001	.020±.015	.301	0.431	0.130±.017	1.598±.012
16	.003	.059	.056±.026	.002	.005	.003±.001	.030±.016	.315	0.492	0.177±.042	1.315±.025
24	.006	.060	.054±.034	.010	.013	.003±.002	.010±.010	.373	0.543	0.170±.037	1.210±.021
	10% Sucrose in Tris Borate buffer pH 8.7										
0	.029	.037	.008±.001	.066	.075	.009±.004	.019±.015	.072	0.088	0.016±.006	0.810±.055
2	.071	.075	.004±.001	.074	.035	.000±.000	.019±.015	.086	0.573	0.487±.059	0.890±.015
4	.026	.046	.020±.040	.084	.068	.000±.000	.021±.019	.120	0.692	0.572±.145	1.070±.031
8	.027	.032	.005±.000	.014	.034	.020±.020	.020±.018	.261	2.000	1.739±.037	1.320±.032
16	.035	.044	.009±.005	.091	.108	.017±.033	.010±.010	.706	1.800	1.094±.191	1.210±.040
24	.043	.054	.011±.001	.012	.016	.004±.002	.012±.010	.977	1.817	0.840±.080	1.107±.023

Table 36A: Amy^{null} and Amy^{1-a} egg-to-adult viability on media containing 6% Brewer's yeast and various concentrations of sucrose (0%, 1%, 2%, 4% and 8%). At time zero, 100 eggs were transferred into each medium.

Time in Days	Amy^{null} viability					Amy^{1-a} viability				
	Sucrose Concentration					Sucrose Concentration				
	0%	1%	2%	4%	8%	0%	1%	2%	4%	8%
15	34	36	7	41	20	35	1	0	0	4
20	6	14	21	2	21	1	40	38	68	24
25	0	0	0	0	0	0	4	8	0	2
Total	40%	50%	28%	43%	41%	36%	45%	46%	68%	30%

Table 36B: *Amy*^{null} and *Amy*$^{1-a}$ egg-to-adult viability (in a monoculture) on food media containing 3% Brewer's yeast and various concentrations of sucrose (0%,1%,2%,4%,and8%).At time zero, 100 eggs were transferred into each medium

Time in Days	*Amy*^{null} Viability — Sucrose Concentration					*Amy*$^{1-a}$ Viability — Sucrose Concentration				
	0%	1%	2%	4%	8%	0%	1%	2%	4%	8%
15	33	39	8	3	28	28	1	0	0	28
20	13	10	52	7	26	12	8	42	0	4
25	2	0	3	0	1	1	0	13	0	0
Total	48%	49%	63%	10%	55%	41%	9%	55%	0%	32%

Table 36C: *Amy*^{null} and *Amy*$^{1-a}$ egg-to-adult viability (in a monoculture) on food media containing 2% Brewer's yeast and various concentrations of sucrose. At time zero, 100 eggs were transferred into each food medium.

Time in Days	*Amy*^{null} Viability — Sucrose Concentration					*Amy*$^{1-a}$ Viability — Sucrose Concentration				
	0%	1%	2%	4%	8%	0%	1%	2%	4%	8%
1										
15	0	0	0	0	0	0	0	0	0	0
20	0	2	15	7	0	0	0	0	8	11
25	0	15	5	9	32	0	0	0	3	10
Total	0%	17%	20%	16%	32%	0%	0%	0%	11%	21%